

Directory of
Microbicides
for the Protection of Materials
A Handbook

Edited by
Wilfried Paulus

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Printed in the Netherlands

*To Holde Paulus, my wife
whose tolerance made this book a reality*

Preface

This new book is chiefly intended for those who are using microbicides for the protection of materials and processes. Another purpose is to inform teachers and students working on biodeterioration and to show today's technical standard to those engaged in R & D activities in the microbicide field.

The effective use of microbicides presupposes knowledge of their characteristics, e.g. chemical and physical properties, effectiveness and spectrum of efficacy. The properties have to be suited to the intended application to avoid detrimental effects on the properties and the quality of the material to be protected; also production processes in which microbicides are used to avoid disturbances by microbial action must not be disturbed by the presence of those microbicides. Microbicides which discolour materials or cause unpleasant smells, which delay the drying of coatings or tend to spoil the surface quality of coatings have proved unsuitable for certain applications. The same is true for microbicides which decompose when the materials are processed. In processing water, surface-active and corrosive microbicides tend to disturb the normal cycle of production. The effective concentration of preservatives for the in-can/in-tank protection of aqueous functional fluids must not cause any change in the viscosity of the liquids. The requisite microbicide concentration depends on the resistance of the microbes to be inhibited. The range of effectiveness is another important factor to be considered. It is necessary to choose microbicides with the proper effective spectrum; a full range covering all species of microbes is not always a must. Among the characteristics of microbicides which one has to mind unconditionally, ranks their toxicity (the potential to cause harm) which represents a certain hazard. How far the application of a microbicide represents a risk to human health and environment emanating from the hazard depends on the way a microbicide is applied. In general risks can be reduced either by using a microbicide having a low potential to cause harm or by applications leading to low exposures. Human toxicity of microbicides used today in the meantime has been tested extensively and the corresponding depth of experience and knowledge has led to health and safety profiles which make applications of microbicides possible without damage to human health, if the adequate legislative regulations are observed.

Environmental legislation has also a big influence on the current and future use of many chemicals. Biocides/microbicides are in the foreground of discussion. Specific issues such as biodegradability, environmental persistence and toxicity to aquatic organisms are focused on them. The legislation gap in this field has shrunk rapidly in the past few years and proposed legislation in the USA and Europe is already broadly equivalent. Worldwide agreement has not yet been reached, is, however, aimed at and should no longer be an utopia.

The present book is intended to inform about all these issues and to form a comprehensive reference source on any given topic. It is divided in two parts. In Part One 22 contributions of worldwide selected experts present an extensively diversified information about:

- microbicides with regard to the relationship between chemical structure and mode of action and activity
- research and development in consideration of registration procedures
- the above mentioned legislative aspects
- the use of microbicides in the major application areas (17) which are described in detail

The microbicide data are organized in Part Two of the book. When trying to classify, or to subclassify, material-protecting microbicides according to their mode of action e.g. as membrane-active and electrophilic-active ingredients, it turned out that a clear assignment is not always possible. For that reason the editor has resorted to chemistry's principle of classifying according to groups of substances (e.g. alcohols, aldehydes, ketones, acids, esters, amides. etc.), thus providing the first necessary information about the microbicides' properties. The description of the various substance classes (21) generally starts with an outline of the mode and mechanisms of action of the microbicides belonging to the corresponding substance class.

These explanations elucidate that the new book is not a simple revised new edition (second) of the handbook 'Microbicides for the Protection of Materials' which appeared 1993. 'Part Two – Microbicide Data' of the new book in the main is an actual inventory of the 'old' book, whereas Part One signifies an important perfection and increase in actuality.

W.P.

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As editor I have to thank the Publishing Managers of Kluwer Academic Publishers, who put up with delays in completion of the book. I would also especially like to thank all the contributing authors, those that met the early deadlines and those that I repeatedly reminded and finally came through with their chapters. Whilst blending the individual contributions into a unified whole the individuality of style has been retained. I trust that this multi-faced approach to the topic will make the book particularly useful to all those are concerned with microbicides for the protection of materials and processes.

The editor wishes also to thank the microbicide suppliers who provided information on their products. Thanks are due to the staff of Bayer Chemical - Material Protection Products for their help - particularly Ulrich Beck and Otto Exner for their encouragement and assistance in the preparation of the book. Acknowledgement is also due to three women of Bayer Chemicals, MPP-TM Christel Born, Annette van de Loo, Heike Aust – who worked with me on the book planning and editing and bore the brunt of my frustrations and anxieties during the whole affair.

W.P.

Note from the Editor

The book presents a comprehensive discussion of the most common microbicides (approx. 300) used for the protection of materials and processes against biodeterioration. The characteristics of each microbicide, e.g. chemical and physical properties, effectiveness and spectrum of efficacy, the knowledge of which is a prerequisite for the effective use of microbicides, are discussed. It is also informed about the toxicity and ecotoxicity of the microbicides. The corresponding depth of knowledge and experience makes uses of microbicides possible without damage to human health and environment. The corresponding legislative aspects are regarded in a special chapter.

This new book is not a simple revised new edition (second) of the Handbook Microbicides for the Protection of Materials which appeared in 1993.

This edition is divided into two parts. In Part One 22 contributions of worldwide selected experts present extensively diversified information about:

- Microbicides with regard to the relationship between chemical structure and mode of action and activity
- Research and development in consideration of registration procedures
- Legislative aspects
- The use of microbicides in the major application areas (17) which are described in detail.

The Microbicide Data are organized in 21 substance classes (e.g. alcohols, aldehydes, acids, amides, etc.) and collected in Part Two.

Part One signifies an important perfection and increase in actuality. Part Two-Microbicide Data is an eagerly anticipated actual inventory of the old book. The combination of the two parts in one book is special and has resulted in the most authoritative information in the field. Accordingly this book will be invaluable not only to all those using microbicides, but also to teachers and students working in biodeterioration and anyone engaged in research and development in the microbicide field.

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Contents

Part One—Microbicides

1	Introduction to microbicides			
	W. PAULUS	3		
1.1	Micro-organisms-microbicides	3	4.1.2	Master plan wanted
1.2	Evaluation of preservatives	7	4.1.3	The enigma of classification
	References	8	4.1.4	A dialogue with a registrant
			4.1.5	Conclusion
				References
				Glossary
2	Relationship between chemical structure and activity or mode of action of microbicides		4.2	The European biocidal products directive
	W. PAULUS	9		B. SCHMIDT-SONNENSCHNEIN
2.1	Introduction	9	4.2.1	Introduction
2.2	Classification of microbicides	10	4.2.2	Content of the biocidal products directive
2.2.1	Membrane-active microbicides	10		Definition of a biocidal product
2.2.2	Electrophilically active microbicides	13		Scope
2.3	Microbial resistance to microbicides	20		Authorisation for placing on the market of biocidal products
2.4	Effectiveness of microbicides and the duration of their effect	21		Frame formulations
2.5	Summary	22		Mutual recognition
	References	22		Provisional authorisation
				Inclusion of an active substance in Annex I or IA
				The substitution principle
				Data protection
				Research and development
				Confidentiality
				Classification, packaging and labelling of biocidal products
				Advertising
				Poison control
				The Annexes
				Summary
				4.2.3
				Transitional measures and the review programme
				4.2.4
				The technical guidance documents
				References
3	R&D in material protection: new biocides		4.3	Protection of health – Microbicides in the environment
	R. BRUNS, J. KAULEN, O. KRETSCHIK, M. KUGLER and H. UHR	25		C. MACKIE
3.1	Introduction: situation of R&D in material protection	25	4.3.1	Introduction
3.2	Fungicides	25	4.3.2	Components of a risk assessment
3.2.1	Azoles [II, 14.]	26	4.3.3	Risk assessment of physico-chemical properties
3.2.2	Multiside inhibitors	27		Risk characterisation on grounds of explosivity, oxidising properties and flammability
3.2.3	Chelating agents	29	4.3.4	Risk assessment for human health
3.3	Bactericides	30		Health effects assessment and identification of adverse effects
3.3.1	Membrane-active compounds	30		Exposure assessment
3.3.2	Electrophilic substances	31		Risk characterisation
3.3.3	Chelating agents	33	4.3.5	Risk assessment for the environment
3.3.4	Inorganic bactericides	34		Ecotoxicity effects assessment
3.4	Insecticides	34		Effects of secondary poisoning
3.4.1	Pyrethroids	34		Exposure assessment
3.4.2	Insect growth regulators	35		Risk characterisation
3.4.3	Chloronicotinyle	37	4.3.6	Overall conclusions
3.4.4	Phenylpyrazole	37		References
3.5	Antifouling	38		
3.5.1	Copper and organic biocides	38		
3.5.2	Natural toxins	39		
3.5.3	Other concepts	40		
3.6	New approaches	41		
3.6.1	Antimicrobial enzymes	41		
3.6.2	Biofilms	41		
3.7	New technologies in research	42		
3.8	Testing of new biocides	43		
3.8.1	Screening for new fungicides	43		
3.8.2	Screening for new Antibacterials	43		
3.8.3	Screening for new insecticides	44		
3.8.4	Screening for new actives against marine antifouling	44		
3.8.5	High throughput screening	44		
3.9	Conclusion	44		
	References	45		
4	Legislative aspects		5	Fields of application
4.1	United States antimicrobial pesticide regulations		5.1	Efficacy of biocides against biofilms
	S.C. OSLOSKY and D. PAWELLEK	47		S. SCHULTE, J. WINGENDER and H.-C. FLEMMING
4.1.1	Introduction: Pesticides beyond the agricultural application	47	5.1.1	Characteristics of biofilms

5.1.2	Definitions of antimicrobial agents	96	5.3.3.4	Chlorine	154
5.1.3	Resistance of biofilm organisms to biocides	97	5.3.3.5	Copper compounds	154
5.1.3.1	Influence of abiotic factors	97	5.3.3.6	Sodium bromide	154
5.1.3.2	Enhanced resistance of biofilms	97	5.3.3.7	Silver	154
5.1.3.3	Transport limitation by reaction-diffusion interaction	98	5.3.4	Oxidizing agents	154
5.1.3.4	Slow growth rate and general stress response	99	References		155
5.1.3.5	Role of biofilm-specific phenotype	100			
5.1.3.6	Role of persister cells	100			
5.1.4	Methods to study antimicrobial action on biofilms	101	5.4 Oilfield application for biocides		
5.1.4.1	Planktonic assays	101	D.B. McILWAINE		157
5.1.4.2	Surface Contamination experiments	101	5.4.1	Introduction	157
5.1.4.3	Biofilm experiments	101	5.4.2	Description of an oilfield	157
5.1.5	Selected biocides	104	5.4.2.1	The injection system	158
5.1.5.1	Chlorine-containing compounds [II, 21.2]	104	5.4.2.2	The production system	158
5.1.5.2	Ozone	107	5.4.3	Oilfield microbiology	160
5.1.5.3	Peroxygens	107		Sulfate reducing bacteria	161
5.1.5.4	Silver compounds	113		Iron and manganese oxidizing bacteria	161
5.1.5.5	Surface-bound biocides and "activated surfaces"	114		Acid producing bacteria	161
5.1.6	Conclusions	115		Biofilms	161
Bibliographic references		115		Problems caused by biofilms	162
			5.4.4	Commonly used oilfield biocides	162
			5.4.4.1	Acrolein [II, 2.6.]	162
			5.4.4.2	Bronopol (II, 17.14.)	163
			5.4.4.3	2,2-Dibromo-3-nitrilopropionamide (DBNPA) [II, 17.5.]	163
			5.4.4.4	Formaldehyde [II, 2.1]	164
			5.4.4.5	Glutaraldehyde [II, 2.5.]	164
			5.4.4.6	Quaternary ammonium compounds (QACs) [II, 18.1.]	166
			5.4.4.7	THPS [II, 3.6.]	166
			5.4.5	The role of biocides in oil and gas operations	168
			5.4.5.1	Water based drilling muds	169
			5.4.5.2	Completion and workover fluids	169
			5.4.5.3	Fracturing fluids	170
			5.4.5.4	Packer fluids	170
			5.4.5.5	Hydrotest fluids	171
			5.4.6	Biofouling control	171
			5.4.6.1	Water injection and production systems	171
			5.4.7	Alternatives to biocide treatments to control souring	173
				The use of nitrate and nitrite	173
				Sulfate removal by reverse osmosis membranes	173
				Irradiation	173
				The use of anthraquinone	173
			Acknowledgements		174
			References		174
5.2 Microbiological control in cooling water systems			5.5 A review of the microbiological degradation of fuel		
M. LUDENSKY	121		J.A. ROBBINS and R. LEVY		177
5.2.1	Introduction	121	5.5.1	Introduction	177
5.2.2	Cooling water systems	121	5.5.2	Historical background	177
5.2.3	Problems caused by biofouling	122	5.5.3	Fuel distribution system	177
	Biofilms	122	5.5.4	Fuel types and their susceptibility to microbial contamination	178
	Legionella	123		Petroleum fuel	178
	Algae	124		Synthetic fuels	180
	Fungi	124		Biodiesel fuels	180
	Macrofouling	124	5.5.5	Growth requirements for microorganisms	180
5.2.4	Methods of testing and evaluation	125		Water	180
	Identification of planktonic organisms	125		Hydrocarbons – organic nutrients	180
	Biofilm monitoring	125		Oxygen	182
5.2.5	Biocides in cooling water systems	126		Inorganic nutrients	183
	Selection of a biocidal program	126		Temperature	183
	Cooling water biocides	127		Effect of pH	183
	Oxidizing biocides [II, 21.]	127	5.5.6	Microbial contamination in fuel and the fuel distribution system	183
	Non-oxidizing biocides	129		Development of microbial contamination in a fuel storage tank	183
	Synergistic combinations	130		Hormoconis resinae	187
	Control of biofilms with biocides	131		Changes in microbial populations	187
	Discussion	136			
5.2.6	Environmental considerations	136			
	Biocide registration	136			
	Discharge limitations	138			
5.2.7	Current trends in biocide development and applications	138			
References		138			
5.3 Recreational water treatment biocides					
M.J. UNHOCH and R.D. VORE	141				
5.3.1	Introduction	141			
5.3.2	Sanitizers	142			
5.3.2.1	Halogens and hypohalogenites and halogen release compounds [II, 21.2.]	143			
5.3.2.2	Polyhexamethylene biguanide [18.3.3]	150			
5.3.2.3	Silver	151			
5.3.2.4	Iodine [II, 21.2.12]	151			
5.3.2.5	Chlorine generators and supplemental sanitizers	151			
5.3.3	Algicides and fungicides	153			
5.3.3.1	Alkyl dimethyl benzyl ammonium chloride [II, 18.1.2]	153			
5.3.3.2	Poly(oxy)ethylene(dimethylimino)ethylene(dimethylamino)ethylene dichloride [II, 18.1.11]	153			
5.3.3.3	Dodecyl dimethyl ammonium chloride	154			

Significant levels of microbial contamination	188	5.7.6 Conclusions	247
Microbial contamination in subsonic aircraft and their handling systems	188	5.7.7 Acknowledgements	247
Microbial contamination in supersonic aircraft and their handling systems	189	References	248
Marine vessels with water compensated fuel storage systems	189		
5.5.7 Consequences of microbial contamination	189	5.8 Application of microbicides for the storage protection of mineral dispersions	
5.5.8 Signs of microbial contamination	190	P. SCHWARZENTRUBER and P.A.C. GANE	251
5.5.9 Prevention and clean up of microbial contamination	191	Introduction	251
Routine maintenance	191	5.8.1 Pigment manufacturing process	251
Water removal	191	5.8.2 Organic additives for dispersion stabilisation – a rich nutrient basis	252
Industry controls	192	5.8.3 Microbial contamination and its consequences	253
Use of biocides	192	5.8.4 Diversity of bacterial morphologies	254
Tank cleaning	192	5.8.5 Prevention and control of microbial activity – real time monitoring and evaluation	256
Alternate cleaning processes	192	Electronic cell counting: The Coulter-counter	258
5.5.10 Biocides for the protection of fuel storage systems	193	Vital staining	258
Fuel-soluble biocides	194	Novel real-time monitoring and evaluation	258
Water-soluble biocides	194	5.8.6 Constraints on suitable types of microbicides	259
Biocide partitioning	195	2-Bromo-2-nitro-propan-1,3-diol (Bronopol) [II, 17.14.]	259
Aviation fuel biocides	195	Isothiazolin (MIT/CIT/BIT) [II, 15.]	259
5.5.11 Tests to determine microbial contamination in fuel systems	196	Phenol derivatives (e.g. o-phenylphenol) [II, 7.]	260
Fuel sampling	196	Aldehydes (e.g. glutaraldehyde) [II, 2.]	260
Agar medium plating for total viable plate counts	196	Formaldehyde-releasing compounds (e.g. ethyleneglycol-hemiformals) [II, 3.]	260
Dip slide microbial growth detectors	197	3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione (DAZOMET) [II, 3.3.25.]	260
Test kits for the detection of microorganisms	197	2,2-Dibromo-3-nitrilopropionamide (DBNPA) [II, 17.5.]	260
Microscopic observations	198	Methylene bithiocyanate (MBT) [II, 20.9.1.]	261
Immunofluorescence	198	5.8.7 Regulatory, safety and environmental issues – some practical points	261
Catalase determination	198	Biocidal product directive 98/8/EG	261
Biocide efficacy testing	199	Risk for humans and environment	261
5.5.12 Conclusions	199	References	261
Acknowledgements	200		
Bibliographic references	200		
5.6 Microbicides for coolants		5.9 Protection of cosmetics and toiletries	
W. SIEGERT	203	R. SCHOLTYSEK	263
5.6.1 Introduction	203	5.9.1 Introduction	263
5.6.2 Care of coolants	204	5.9.2 Description of cosmetic products	263
5.6.2.1 General	204	5.9.2.1 Definition	263
5.6.2.2 Preservation	204	5.9.2.2 Important product classes	263
5.6.2.3 Preservatives	212	5.9.2.3 Special products of microbiological relevance	264
5.6.3 Summary	217	5.9.3 Microbiological safety of cosmetic products	265
		5.9.3.1 Microbiologically susceptibility	265
5.7 The microbial spoilage of polymer dispersions and its prevention		5.9.3.2 Causes and consequences of microbial contamination	265
J. GILLAT	219	5.9.3.3 Official regulations	267
5.7.1 Introduction	219	5.9.4 Protection of cosmetics from microbial spoilage	269
5.7.2 The manufacture of polymer dispersions	219	5.9.4.1 The concept	269
5.7.2.1 Monomers	219	5.9.4.2 Preservation using physical and chemical factors	269
5.7.2.2 Surfactants	220	5.9.4.3 Influence of cosmetic constituents on product preservation	270
5.7.2.3 Initiators	222	5.9.4.4 Selection of appropriate preservatives	274
5.7.2.4 The manufacturing process	223	5.9.5 Validation of effective preservation	277
5.7.3 Polymer dispersion types and their applications	224	5.9.5.1 Types of primary packaging	277
5.7.4 The microbiology of polymer dispersions	224	5.9.5.2 Preservative challenge test	278
5.7.4.1 Components affecting susceptibility	224	5.9.5.3 In-use tests	281
5.7.4.2 Causative microorganisms	227	5.9.6 Production hygiene	281
5.7.4.3 The effects of microbial contamination	228	5.9.7 Fault prevention analyses	282
5.7.4.4 Sources of microbial contamination	232	5.9.8 Summary	284
5.7.5 Prevention and control of microorganisms in polymer dispersions	235	References	284
5.7.5.1 Avoiding contamination	236		
5.7.5.2 Improving plant hygiene	237		
5.7.5.3 The use of biocides	237		
5.7.5.4 Evaluation of polymer dispersion biocides	246		

5.10 Food and beverage preservation

N.N. RACZEK	287
5.10.1 Introduction	287
5.10.2 Preservatives	288
5.10.2.1 Benzoic acid [II, 8.1.9.]	288
5.10.2.2 Sorbic acid [II, 8.1.5.]	289
5.10.2.3 Propionic acid [II, 8.1.3.]	290
5.10.2.4 Sulfur dioxide, sulfites [II, 8.2.2.]	292
5.10.2.5 Nitrite, nitrate	293
5.10.2.6 Esters of p-hydroxybenzoic acid (parabens) [II, 8.1.11.]	294
5.10.2.7 Nisin [II, 20.11.1.]	295
5.10.2.8 Pimaricin [II, 20.11.2.]	296
5.10.2.9 Antibiotics [II, 20.11.]	297
5.10.2.10 Dimethyldicarbonate (DMDC) [II, 9.7.]	298
5.10.2.11 Dehydroacetic acid [II, 8.1.8.]	299
5.10.2.12 Thiabendazole [II, 15.9.]	299
5.10.2.13 Biphenyl [II, 20.7.]	300
5.10.2.14 o-Phenylphenol [II, 7.4.1.]	301
References	301

5.11 Disinfectants and sanitizers

J. GEBEL, A. KIRSCH-ALTENA, V. VACATA and M. EXNER	305
5.11.1 Introduction	305
5.11.2 Historical review	306
5.11.3 Application fields	307
Human medicine	308
Food, industrial, domestic, and institutional areas	308
Veterinary field	308
5.11.4 Disinfection in the medical field/human medicine	308
Hands/Skin	308
Surfaces	309
Instruments	309
Laundry	310
5.11.5 Efficacy of disinfectants	310
Factors affecting the efficacy of disinfectants	310
Test methods	311
5.11.6 Normative and legislative regulations	311
Relevant laws	313
Regulatory procedures for active ingredients, end-use products, new and old biocides	313
Harmonized CEN standards	314
ISO-Standards	314
USA – EPA and FDA regulations	314
Summary	314
Acronyms and Abbreviations	315
References	315

5.12 Microbicide applications in the leather industry

C. HAUBER	317
5.12.1 Introduction	317
5.12.1.1 Historical development	317
5.12.1.2 The structure of the skin	317
5.12.2 Leather manufacture	318
5.12.2.1 Sensitive process steps	318
5.12.2.2 Damages caused by micro-organisms	318
5.12.2.3 Preservation of the raw hide	321
5.12.2.4 Preservation of semi-processed leather	321
Acknowledgments	323
References	324

5.13 Microbial degradation of plastics

P. DYLINGOWSKY and R.G. HAMEL	325
5.13.1 History of plastics and biocides for plastics	325
5.13.2 Susceptibility of plastics	326
Flexible PVC	326
Plasticizers	326
Other additives	327
Polyurethanes	327
TPE/TPR	327
Other plastics	328
Applications	328
5.13.3 Cosmetic degradation	328
Responsible organism	328
Staining of flexible PVC	329
5.13.4 Structural degradation of plastics	332
Action of enzymes on plastics	333
5.13.5 Characteristics of a good biocide for plastics	333
Spectrum of activity against microorganisms	333
Compatibility	333
Dispersibility	333
Heat stability	333
Weatherability	334
Toxicity	334
Physical form	334
5.13.6 Evaluation of biocides in plastics	334
Microbiological tests	334
Petri dish methods – bacteria/actinomycetes	334
Petri dish methods – actinomycetes/bacteria (nutrient media)	335
Petri Dish Methods – Fungi (non-nutrient media)	336
Quantitative test for bacteria	337
Soil burial test – appendix G	338
Algal resistance – appendix H	339
Humidity chamber test – appendix I	339
Outdoor exposure	340
Regulatory	341
Bibliographic references	342
Appendix	342

5.14 Surface coatings

W. LINDNER	347
5.14.1 Introduction	347
5.14.2 Architectural coatings	348
5.14.2.1 Classification of architectural paints	348
5.14.2.2 Environmental regulation	350
5.14.2.3 Microbicides in paints – historical background	350
5.14.3 Today's microbicides in the paint industry	352
5.14.3.1 In-can preservation	352
5.14.3.2 In-can preservatives	354
5.14.3.3 Test methods	359
5.14.3.4 Plant and production hygiene	360
5.14.5 Film preservation (dry film protection)	360
5.14.5.1 Microbial defacement of interior coatings	361
5.14.5.2 Interior film preservatives	363
5.14.5.3 Microbial defacement of exterior coatings	363
5.14.5.4 Exterior film preservatives	367
5.14.5.5 Film preservation test methods	371
5.14.6 Antifouling coatings	372
5.14.7 Summary	373
References	374

5.15 Pulp & paper

G. CORBEL	377
5.15.1 Introduction	377
5.15.1.1 Problems due to micro-organisms	378

5.15.1.2	Microorganisms common to papermaking systems	379	5.16.3	Voluntary safety – and environmental labels	412
5.15.1.3	Biological deposition on paper machines	382	5.16.4	Test methods	412
5.15.1.4	Goal of microbial control programs	384	5.16.5	Preservation of textile materials by proper maintenance	413
5.15.2	Control of microbiological growth in the paper industry	384	5.16.6	Preservation of textile materials by anti-microbial modification	415
5.15.2.1	Non oxidising biocides in paper making	384	References		417
5.15.2.2	Oxidising biocides [II, 21.] in paper making	389	5.17 Industrial wood protection		
5.15.2.3	Bio-dispersants on short loop treatment of paper machines	393	G.R. WILLIAMS		419
5.15.2.4	Enzymatic slime control	393	5.17.1	Foreword	419
5.15.2.5	Mold proofing treatment of finished paper	394	5.17.2	Wood as a raw material	419
5.15.2.6	Catalase control in papermaking	395	5.17.2.1	Wood structure and chemistry and its importance in wood protection	419
5.15.3	Monitoring microbial populations and screening efficacy of biocontrol agents	395	5.17.3	Agencies of deterioration and their occurrence	421
5.15.3.1	Lab analysis	395	5.17.3.1	Degrade of wood by insects	421
5.15.3.2	Bioaudit	396	5.17.3.2	Bacterial degrade of wood	422
5.15.3.3	Biocide screening methods	397	5.17.3.3	Fungal degrade of wood	423
5.15.3.4	Catalase test	397	5.17.3.4	Marine borer damage to wood	425
5.15.3.5	Microscope examination	397	5.17.4	Methods of assessment for wood preservatives	426
5.15.3.6	Oxido-reduction potential	398	5.17.4.1	Stain and mould fungi	426
5.15.3.7	Hydrogen sulphide measurement	400	5.17.4.2	Laboratory fungal decay testing	427
5.15.3.8	ATP based monitoring tests	401	5.17.4.3	Insect testing in the laboratory	427
5.15.3.9	Optical fouling monitor	402	5.17.4.4	Field evaluation methodologies	428
5.15.3.10	Microbiological growth media	403	5.17.5	Wood preservative formulations	430
5.15.3.11	Volatile fatty acid (VFA) measurement	404	5.17.5.1	Industrial application of wood preservative formulations	430
5.15.4	Equipment	404	5.17.5.2	Wood preservative design	430
5.15.5	Safety	406	5.17.5.3	Metal-based wood preservative systems	432
5.15.5.1	Safe handling of biocides	406	5.17.5.4	Organic wood preservative chemicals	433
5.15.5.2	Developing a safe microbiological control program	407	5.17.5.5	Insecticide components of wood protection formulations	436
5.15.5.3	Disposal issues	408	5.17.6	Health, safety and the environment	437
5.15.5.4	Program application approvals/ registration [I, 4.]	408	5.17.6.1	Restriction on the use of active ingredients	437
5.16 Microbicides for the protection of textiles			5.17.6.2	Availability of active ingredients	437
A.W. WYPKEMA		411	5.17.6.3	Effect on treatment process	438
5.16.1	Definition and differentiation of terms	411	5.17.6.4	Specification of wood preservatives	438
5.16.2	Legislation	411	5.17.6.5	Development of methods for the determination of environmental impact	438
			5.17.7	Summary comments	438
			References		438

Part Two—Microbicide data

Organisation of microbicide data

1. ALCOHOLS	444	2.3.	Glyoxal	464	
1.1.	Ethanol	444	2.4.	Succinaldehyde	465
1.2.	1-Propanol	445	2.5.	Glutaraldehyde	465
1.3.	2-Propanol	446	2.6.	2-Propenal	468
1.4.	Benzyl alcohol	447	2.7.	Chloroacetaldehyde	469
1.5.	2,4-Dichlorobenzyl alcohol	448	2.8.	alpha-Bromocinnamaldehyde (BCA)	470
1.6.	2-Phenyl-ethan-1-ol	449	2.9.	3,5-Dichloro-4-hydroxy-benzaldehyde (DCHB)	471
1.7.	2-Phenoxy-ethan-1-ol	450	2.10.	2-Hydroxy-1-naphthaldehyde (HNA)	472
1.8.	3-Phenyl-propan-1-ol	452	2.11.	Phthalic dialdehyde	473
1.9.	1-Phenoxy-propan-2-ol	452			
1.10.	1,1,1-Trichloro-2-methyl-2-propanol	453	3. FORMALDEHYDE RELEASING COMPOUNDS	474	
1.11.	3-Iodo-2-propin-1-ol (IPA)	454	3.1.	O-Hydroxymethyl Compounds (hemiformals and formals)	474
1.12.	1,1,2-Triiodo-propene(1)-3-ol	455	3.1.1.	1-Butanolhemiformal	476
1.13.	1,2-Ethanediol	456	3.1.2.	Benzylalcoholmono(poly)-hemiformal (BHF)	476
1.14.	1,2-Propanediol	457	3.1.3.	2-Phenoxyethanolhemiformal	478
1.15.	1,3-Butanediol	457	3.1.4.	Ethyleneglycolhemiformals and ethyleneglycolformal = 1,3-dioxolane	479
1.16.	2-(Butoxyethoxy)ethanol	458	3.1.5.	(2-(2-butoxyethoxy)ethoxy)methanol	481
1.17.	3-(4-Chlorophenoxy)-1,2-propanediol	459	3.1.6.	(3-Iodopropargyl)-(4-chlorophenyl) formal	482
2. ALDEHYDES	459				
2.1.	Formaldehyde	460			
2.2.	Acetaldehyde	463			

3.2.	C-Hydroxymethyl Compounds	482	3.4.11.	Sodium N-hydroxymethyl-N-methyldithiocarbamate	525
3.2.1.	Tris(hydroxymethyl)nitromethane	483	3.4.12.	1-Hydroxymethyl-2-thiono-1:2-dihydro-benzothiazol	525
3.2.2.	Bis(hydroxymethyl)-trifluoromethyl-nitromethane	485	3.4.13.	3-Hydroxymethyl-5,6-dichloro-benzoxazolinone	526
3.2.3.	Mixture of 70% N-(2-nitrobutyl)-morpholin (A) and 20% N,N'-(2-ethyl-2-nitrotrimethylene)-dimorpholine (B)	485	3.4.14.	3-Hydroxymethyl-5-chloro-benzoxazoline-2-thione	527
3.3.	Amine-formaldehyde-reaction-products	487	3.5.	Reaction Products Of Amino Acids With Formaldehyde	528
3.3.1.	Hexamethylenetetramine (HTA)	489	3.5.1.	Sodium 2-hydroxymethylaminoacetate	528
3.3.2.	1-(3-Chloroallyl)-3,5,7-triaza-1-azoniaadamantanechloride	490	3.5.2.	4,4'-Methylenebis-(1,2,4-thiadiazine-1,1-dioxide)	529
3.3.3.	1,1'-(2-Butenylene)-bis-(3,5,7-triaza-1-azoniaadamantane chloride)	491	3.6.	Bis(tetrakis(hydroxymethyl)phosphonium) sulfate	529
3.3.4.	1-Carboxymethyl-3,5,7-triaza-1-azoniaadamante chloride	492			
3.3.5.	1-Carbamoylmethyl-3,5,7-triaza-1-azoniaadamante chloride	493	4.	ACETALDEHYDE RELEASING COMPOUND	531
3.3.6.	1-[(N-hydroxymethyl-carbamoyl)-methyl]-3,5,7-triaza-1-azoniaadamantane chloride	493	4.1.	6-Acetoxy-2,4-dimethyl-1,3-dioxane	531
3.3.7.	Other quaternary hexaminium salts	494	5.	SUCCINALDEHYDE RELEASING COMPOUND	532
3.3.8.	2-[(Hydroxymethyl)amino]alkanols	495	5.1.	2,5-Dimethoxytetrahydrofuran	532
3.3.9.	3, 3'-Methylenebis(5-methyl-1,3-oxazolidine)	497	6.	2-PROPENAL-RELEASING-COMPOUND	533
3.3.10.	4,4-Dimethyl-1,3-oxazolidine	499	6.1.	Copolymer, base: 2-propenal (2.6.) and propane-1,2-diol (1.14.)	533
3.3.11.	(5-Methyl-3-oxazolidinyl)-isopropanol-bis-hemiformal	500	7.	PHENOLICS	534
3.3.12.	(5-Ethyl-3,7-dioxo-1-azabicyclo(3.3.0.)-octane	501	7.1.	Phenol	537
3.3.13.	5-Hydroxymethyl-1-aza-3,7-dioxabicyclo (3.3.0) Octane	503	7.2.	Alkylphenols	538
3.3.14.	Polymethoxy Bicyclic Oxazolidines	503	7.2.1.	3,5-Dimethylphenol	538
3.3.15.	Tetrahydro-1,3-oxazine	504	7.2.2.	2-Isopropyl-5-methylphenol	539
3.3.16.	Bis-(tetrahydro-,3-oxazin-3-yl)methane	505	7.2.3.	4-Isopropyl-3-methylphenol	540
3.3.17.	N-ethyl-dihydro-1,3,5-dioxazine	505	7.2.4.	5-Isopropyl-2-methylphenol	541
3.3.18.	Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine	506	7.2.5.	4-(2-Methylbutyl)phenol	542
3.3.19.	Hexahydro-1,3,5-triethyl-s-triazine (HTT)	508	7.2.6.	Benzylphenols	543
3.3.20.	Hexahydro-1,3,5-tris[(tetrahydro-2-furanyl)-methyl]-s-triazine	509	7.2.7.	Cyclohexylphenols	544
3.3.21.	N-Methylene-cyclohexylamine	510	7.3.	Halogenated Alkylphenols	545
3.3.22.	N,N'-methylene-bismorpholine	510	7.3.1.	4-Chloro-3-methylphenol	545
3.3.23.	1.4.6.9-Tetraaza-tricyclododecane (4.4.1.14.9)	511	7.3.2.	4-Chloro-3,5-dimethylphenol	548
3.3.24.	3,5-Dimethyl-1-hydroxymethyl-pyrazole	512	7.3.3.	2,4-Dichloro-3,5-dimethylphenol	549
3.3.25.	Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione	513	7.3.4.	4-Chloro-6-isopropyl-3-methylphenol	550
3.3.26.	5-Amino-1,3-bis(2-ethylhexyl)-5-methyl-hexahydropyrimidine	514	7.3.5.	2-Benzyl-4-chlorophenol	551
3.4.	Amide-formaldehyde-reaction-products	515	7.3.6.	4-Chloro-2-cyclopentylphenol	553
3.4.1.	N-Hydroxymethyl-chloracetamide	516	7.3.7.	2-Methyl-3,4,5,6-tetrabromophenol	554
3.4.2.	2,2,3-Trichloro-N-hydroxymethyl-propionamide	517	7.4.	Biphenylols	555
3.4.3.	N-hydroxymethyl-ureas	518	7.4.1.	2-Biphenylol	555
3.4.4.	N,N'-bis(hydroxymethyl)thiourea	518	7.4.2.	4-Biphenylol	558
	N-hydroxymethyl-S-hydroxymethyl-thiourea	518	7.4.3.	4-Chloro-2-hydroxybiphenyl	559
3.4.5.	N-hydroxymethyl-N'-methyl-thiourea	519	7.5.	Halogenated Phenols	560
3.4.6.	N-(hydroxymethyl)-N-[1,3-bis(hydroxymethyl)-2,5-dioxo-imidazolidin-4-yl]-N'-hydroxymethyl-urea	519	7.5.1.	4-Chlorophenol	560
3.4.7.	Bis-(N'-hydroxymethyl-2,5-dioxoimidazolidin-4-yl)-ureido-methane	521	7.5.2.	2,4,5-Trichlorophenol	561
3.4.8.	1-(Hydroxymethyl)-5,5-dimethyl-2,4-dioxo-imidazolidine	522	7.5.3.	2,4,5-Trichlorophenol	562
3.4.9.	1,3-Bis(hydroxymethyl)-5,5-dimethyl-2,4-dioxoimidazolidine	522	7.5.4.	Pentachlorophenol (PCP)	563
3.4.10.	1,3,4,6-Tetrakis-hydroxymethyl-tetrahydroimidazo-(4,5-D)-imidazole-2,5-dione	524	7.5.5.	2,4,6-Tribromophenol	564
			7.6.	Phenoxyphenols	564
			7.6.1.	5-Chloro-2-(2,4-dichlorophenoxy)phenol	564
			7.6.2.	5-Chloro-2-(4-chlorophenoxy)phenol	566
			7.7.	Bisphenols	567
			7.7.1.	Bis-(4-hydroxyphenyl)-methane	567
			7.7.2.	2,2-Bis(4-hydroxyphenyl)propane	568
			7.7.3.	2,2'-Methylenebis (4-chlorophenol)	569
			7.7.4.	2,2'-Methylenebis (3,4,6-trichlorophenol)	570

7.7.5.	2,2'-Methylenebis(6-bromo-4-chlorophenol)	571	11.9.	3-(3-Iodopropargyl)-6-chlorobenzoxazol-2-one	626
7.7.6.	2,2'-Thiobis(4-chlorophenol)	572	11.10.	Salts of N-Methylthiocarbamic acid	627
7.7.7.	2,2'-Thiobis(4,6-dichlorophenol)	573	11.10.1.	Sodium N-methylthiocarbamate	627
7.8.	Nitrophenols	573	11.10.2.	Potassium N-methylthiocarbamate	627
7.8.1.	4-Nitrophenol	574	11.11.	Salts Of N,N-Dimethylthiocarbamic Acid	628
8.	ACIDS	574	11.11.1.	Sodium dimethylthiocarbamate	628
8.1.	Organic Acids	575	11.11.2.	Potassium dimethylthiocarbamate	629
8.1.1.	Formic acid	575	11.11.3.	Zinc dimethylthiocarbamate	630
8.1.2.	Acetic acid	576	11.12.	Salts Of Ethylene-1,2-Bisdithiocarbamic Acid	632
8.1.3.	Propionic acid	577	11.12.1.	Disodium ethylene-1,2-bisdithiocarbamate	632
8.1.4.	DL-Lactic acid	578	11.12.2.	Zinc ethylenebisdithiocarbamate	632
8.1.5.	Sorbic acid	579	11.13.	Bis(Alkylthiocarbamoyl)-Disulphides	633
8.1.6.	n-Octanoic acid	580	11.13.1.	Bis(dimethylcarbamoyl)disulphide	633
8.1.7.	Undec-10-enoic acid	580	12.	DIBENZAMIDINES	634
8.1.8.	Dehydroacetic acid (DHA)	581	12.1.	4,4'-Diamidinophenoxypropane	635
8.1.9.	Benzoic acid	583	12.2.	4,4'-Diamidino-2,2'-dibromodiphenoxypropane	636
8.1.10.	Salicylic acid	585	12.3.	4,4'-(Hexamethylenedioxy)dibenzamidine	637
8.1.11.	Alkyl 4-hydroxybenzoates	586	12.4.	4,4'-Diamidino-2,2'-dibromodiphenoxyhexane	638
8.1.12.	Naphthenic acid, tech.	593	13.	PYRIDINE DERIVATIVES AND RELATED COMPOUNDS	638
8.1.13.	n-Dodecanoic acid	594	13.1.	Pyridine-N-oxides	639
8.2.	Inorganic Acids	595	13.1.1.	2-Hydroxypyridine N-oxide	639
8.2.1.	Boric acid	595	13.1.2.	1-Hydroxy-4-methyl-6-(2,4,2-trimethylpentyl)-2(1H)pyridone ethanolamine salt	640
8.2.2.	Sulphurous acid anhydride = Sulphur dioxide	597	13.1.3.	2-Thiolpyridine N-oxide	641
9.	ACID ESTERS	598	13.1.4.	2,2'-Dithio-bis(pyridine-N-oxide)	646
9.1.	Ethyl formate	598	13.2.	Pyridine-4-carboxylic acid hydrazide	647
9.2.	Ethyl bromoacetate	599	13.3.	8-Quinolinol	647
9.3.	Benzyl bromoacetate	600	14.	AZOLES	650
9.4.	1,2-Bis(bromoacetoxy) ethane	600	14.1.	α -[2-(4-Chlorophenyl)ethyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazolyl-(1)-ethanol	651
9.5.	1,4-Bis(bromoacetoxy)-2-butene, (BBAB)	601	14.2.	1-[(2-(2',4'-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl)-1H-1,2,4-triazole	652
9.6.	1-Bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene	602	14.3.	1-[2-(2,4-Dichlorophenyl)-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole	653
9.7.	Dimethyl dicarbonate (DMDC)	603	14.4.	α -(4-Chlorophenyl)- α -(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol	654
9.8.	Glyceryl monolaurate (α - and β -form)	605	14.5.	1-[2-(2,4-Dichlorophenyl)-2-(2-propenyloxy)ethyl]-imidazole	655
9.9.	Dodecanoic acid pentachlorophenyl ester	606	15.	HETEROCYCLIC N, S COMPOUNDS	657
9.10.	Fatty acid esters (mix.) of 5,5'-dichloro-2,2'-dihydroxydiphenylmethane	606	15.1.	2-Methyl-4-isothiazolin-3-one (MI)	657
9.11.	2,2'-[(1,1,3-trimethyl-1,3-propanediyl)bis(oxy)]bis[4,4,6-trimethyl-1,2,3-dioxyborinane]	607	15.2.	5-Chloro-2-methyl-4-isothiazolin-3-one (CMI)	658
10.	AMIDES	608	15.3.	Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (15.2.) and 2-methyl-4-isothiazolin-3-one (15.1.)	659
10.1.	Salicylamide	609	15.4.	2-n-Octyl-4-isothiazolin-3-one (OI)	661
10.2.	N-Alkylsalicylamides	609	15.5.	4,5-Dichloro-2-(n-octyl)-4-isothiazolin-3-one (DCOI)	663
10.2.1.	N-Butylsalicylamide	609	15.6.	1,2-Benzisothiazolin-3-one (BIT)	664
10.2.2.	N-Hexylsalicylamide	610	15.7.	N-Butyl-1,2-benzisothiazolin-3-one (BBIT)	666
10.2.3.	N-Octylsalicylamide	610	15.8.	2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)	667
10.2.4.	N-Decylsalicylamide	610	15.9.	2-(1,3-Thiazol-4-yl) benzimidazole (TBZ)	669
10.2.5.	N-Dodecylsalicylamide	610	15.10.	2-Mercaptobenzothiazole (A) $\leftrightarrow$ Benzothiazolin-2- thione(B)	670
10.2.6.	N-Tetradecylsalicylamide	610	15.11.	2-(Thiocyanomethylthio)benzthiazole (TCMBT)	671
10.3.	Salicylanilide	610	16.	N-HALOALKYLTHIO COMPOUNDS	672
10.4.	3,4',5-Tribromosalicylanilide	611	16.1.	N-(Trichloromethylthio)phthalimide	673
10.5.	Dithio-2,2'-bis(benzmethylamide)	612	16.2.	N-(Fluorodichloromethylthio)phthalimide	674
10.6.	N-Cylohexyl-N-methoxy-2,5-dimethyl-3-furan carboxamide	613	16.3.	N-(Trichloromethylthio)cyclohex-4-ene-1,2- dicarboximide	675
10.7.	N-(2-methylnaphthyl)maleinimide	614	16.4.	N-1,1,2,2-Tetrachloroethylthiotetrahydrophthalimide	676
10.8.	N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)urea	615	16.5.	N-N-Dimethyl-N'-phenyl-N'-dichlorofluoromethylthiosulphamide	677
10.9.	N'-(3,4-dichlorophenyl)-N,N-dimethylurea	617			
10.10.	4-Trifluoromethylphenylsulfonic acid amide	618			
11.	CARBAMATES	618			
11.1.	3-Iodopropynylbutylcarbamate (IPBC)	619			
11.2.	3-Iodopropynylphenylcarbamate (IPPC)	620			
11.3.	3-Iodopropynylcarbamate (IPC)	621			
11.4.	Methyl-N-(2-benzimidazolyl)carbamate	622			
11.5.	Methyl-N-(1-butylcarbamoyl)- benzimidazol-2-ylcarbamate	623			
11.6.	3-Butyl-2,4-dioxo-s-triazino[1,2-a]-benzimidazole (STB)	624			
11.7.	5,6-Dichlorobenzoxazolinone	625			
11.8.	3-(3-Iodopropargyl) benzoxazol-2-one	626			

16.6.	N,N-dimethyl-N'-tolyl-N'-dichlorofluoromethylthiosulphamide	678	19.	ORGANOMETALLIC COMPOUNDS	733
16.7.	N-methyl-N'-3,4-dichlorophenyl-N'-dichlorofluoromethylthiourea	680	19.1.	10, 10'-Oxybisphenoxysarsine (OBPA)	734
17.	COMPOUNDS WITH ACTIVATED HALOGEN ATOMS	681	19.2.	Phenylmercury acetate (PMA)	735
17.1.	2-Chloroacetamide	682	19.3.	Phenylmercury oleate (PMO)	736
17.2.	2-Bromoacetamide	684	19.4.	Sodium ethylmercury thiosalicylate	736
17.3.	2-Iodoacetamide	684	19.5.	Bis(tributyltin) oxide (TBTO)	737
17.4.	N-(4-Bromo-2-methylphenyl)-2-chloroacetamide (BMPCA)	685	19.6.	Tributyltin esters (TBT esters)	739
17.5.	2,2-Dibromo-3-nitrilopropionamide (DBNPA)	686	19.6.1.	Tributyltin benzoate (TBTB)	739
17.6.	2-Bromo-4'-hydroxyacetophenone	689	19.6.2.	Tributyltin salicylate (TBTS)	741
17.7.	Bis(trichloromethyl)sulphone	690	19.6.3.	Tributyltin linoleate (TBTL)	741
17.8.	p-[(Diiodomethyl)sulphonyl]toluene	690	19.6.4.	Tributyltin naphthenate/TBTN)	742
17.9.	p-[(Diiodomethyl)sulphonyl]-chlorobenzene	692	19.6.5.	Other tributyltin carboxylic acid esters	743
17.10.	3,3,4,4-Tetrachloro-tetrahydro-1,1-dioxo-thiophene	692	19.6.6.	Tributyltin fluoride (TBTF)	743
17.11.	(2-Chloro-2-cyanovinyl)-phenylsulphone	693	19.6.7.	Triphenyltin chloride (TPTC)	744
17.12.	2,3,5,6-Tetrachloro-4-(methylsulphonyl)pyridine	694	20.	VARIOUS COMPOUNDS	745
17.13.	2-Bromo-2-nitro-propan-1-ol (BNP)	695	20.1.	N-Cyclohexyl-N'-hydroxy-diazemiumoxide derivatives- HDO salts	745
17.14.	2-Bromo-2-nitropropane-1,3-diol	697	20.2.	2,6-Dimethyl-4-tridecylmorpholine	746
17.15.	5-Bromo-5-nitro-1,3-dioxane	699	20.3.	±-cis-4-[3-(4-tert. Butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine	747
17.16.	2,2-Dibromo-2-nitroacetamide	701	20.4.	N-Cyclopropyl-N'-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine	747
17.17.	(2-Bromo-2-nitroethenyl)-benzene	701	20.5.	2,4-bis(Ethylamino)-6-chloro-1,3,5-triazine	749
17.18.	1,2-Dibromo-2,4-dicyanobutane (DCB)	702	20.6.	2-tert.-Butylamino-6-chloro-4-ethylamino-s-triazine	749
17.19.	2,4,5,6-Tetrachloro-1,3-dicyanobenzene	704	20.7.	Biphenyl	750
17.20.	2,4-Dichloro-6-(2-chloranilino)-1,3,5-triazine	705	20.8.	1-Chloronaphthalene	751
17.21.	Phenylmethanesulphonyl fluoride (PMSF)	706	20.9.	Thiocyanates (R-S-C≡N) and Isothiocyanates(R-N = C = S)	752
17.22.	4,5-Dichloro-3H-1,2-dithiol-3-one	706	20.9.1.	Methylene bis(thiocyanate) (MBT)	752
18.	SURFACE ACTIVE AGENTS	707	20.9.2.	Methyl isothiocyanate	753
18.1.	Quaternary ammonium and phosphonium compounds	709	20.9.3.	Allyl isothiocyanate	754
18.1.1.	Hexadecyltrimethylammonium bromide	710	20.10	Sustainable active microbicidal (SAM) polymers	754
18.1.2.	N-Alkyl(C ₈ -C ₁₈)-N, N-dimethyl-N-benzylammonium chloride = Benzalkonium chloride	711	20.10.1.	Poly(tert.-butylaminoethyl) methacrylate	755
18.1.3.	Diisobutylphenoxyethoxyethyl-dimethylbenzylammonium chloride	712	20.11.	Antibiotics	756
18.1.4.	Di-n-decyl-dimethylammonium chloride (DDAC)	713	20.11.1.	Nisin A	756
18.1.5.	Dioctyl-dimethylammonium chloride	715	20.11.2.	Pimaricin	757
18.1.6.	N-Decyl-N-isononyl-N,N-dimethylammonium chloride	715	21.	OXIDIZING AGENTS	758
18.1.7.	Benzyl-cocoalkyl-dimethylammonium chlorides	716	21.1.	Peroxy Compounds	758
18.1.8.	1-Hexadecylpyridinium chloride monohydrate	717	21.1.1.	Hydrogenperoxid	758
18.1.9.	Dequalinium chloride	718	21.1.2.	Sodium perborate tetrahydrate	759
18.1.10.	3-(Trimethoxysilyl)-propyl-dimethyloctadecylammonium chloride	718	21.1.3.	Peroxyacetic acid	759
18.1.11.	Polymeric Quaternary Ammonium Compounds	720	21.1.4.	Magnesium bis(2-carboxylate-monoperoxybenzoic acid) hexahydrate	760
18.1.12.	Tetraalkylphosphonium compounds (TAPC's)	722	21.1.5.	Higher peroxycarboxylic acids	761
18.2.	Long-Chain Alkylamines	723	21.2.	Halogens, Hypohalogenites and Halogen Releasing Compounds	762
18.2.1.	Dodecylamine	723	21.2.1.	Chlorine	763
18.2.2.	Bis(3-aminopropyl)dodecylamine	724	21.2.2a.	Sodium hypochlorite solution	763
18.2.3.	Fatty amine hydrochlorides (C ₈ -C ₁₈) derived from coconut oil	725	21.2.2b.	Calcium hypochlorite dihydrate	764
18.3.	Guanidines and biguanides	726	21.2.3.	Hypobromous acid	765
18.3.1.	Cocospropylenediamine-1,5-bis-guanidiniumacetate	726	21.2.4.	Chlorine dioxide	766
18.3.2.	Bis(guanidinooctyl)amine triacetate	727	21.2.5.	Chloramine-T	767
18.3.3.	Poly(hexamethylenebiguanide) hydrochloride (PHMB)	727	21.2.6.	Dichloroisocyanuric acid sodium salt dihydrate	767
18.3.4.	1,6-Di(4'-chlorophenyl-diguanido)hexane	729	21.2.7.	Trichloroisocyanuric acid	768
18.4.	Ampholytes	732	21.2.8.	Trichloromelamine (TCM)	769
18.4.1.	Dodecyl-di(aminoethyl)glycine	732	21.2.9.	N-Chlorosuccinimide (NCS)	770
18.4.2.	n-Dodecyl-β-aminopropionic acid	733	21.2.10.	1,3-Dichloro-5,5-dimethylhydantoin (DCDMH)	770
			21.2.11.	1-Bromo-3-chloro-5,5-dimethylhydantoin (BCDMH)	771
			21.2.12.	Poly(vinyl-pyrrolidone) iodine	772
			21.3.	Sodium iodate	773
			REFERENCES	773	
			INDEX	779	

Part One - Microbicides

1 Introduction to microbicides

W. PAULUS

1.1 Micro-organisms – microbicides

Antimicrobial substances will be referred to in this book as ‘microbicides’ in as far as they kill micro-organisms and as ‘microbistats’ in as far as they inhibit the multiplication of micro-organisms. Whether the action of a substance is microbicidal or microbistatic generally depends on the concentration at which the substance is used.

Microbicides belong to the biocides. ‘Biocide’ is a generic term which comprises among others microbicides, molluscicides, acaricides, insecticides, herbicides, rodenticides etc. On the other hand ‘microbicide’ is the generic term for bactericides, fungicides, algicides etc. The experts are fully conversant with these distinctions and take them for granted. Whenever appropriate, however, the same distinctions should be used more widely – not simply to prevent confusion, but also so that the general public is constantly reminded that modern active agents and those undergoing development are not intended to attack the entire ‘bios’, but only a certain limited part of it. Their effects – in other words – are specific; microbicides, for example, are active against micro-organisms.

Micro-organisms in the sense of this book are bacteria, yeasts, fungi, viruses, algae and lichens. The last-mentioned are double organisms consisting of an alga species and a fungus species that vegetate in symbiotic association (symbiosis). The alga uses carbon dioxide, light, water and trace elements to photosynthesize organic material, on which the fungus feeds. For its part, the mycelium of the fungus stores water, which enables the alga to continue living in times of drought.

Microbial cells were the first living cells on earth. They appeared about three and a half thousand million years ago as the starting point of the evolution of life on our planet. Microfossils that have been found in flint and certain other very fine sediments are regarded as direct proof of such early life – of cyanobacteria, for example, which probably formed a blue-green slime in the littoral regions of lakes close to volcanoes.

Micro-organisms, whose cells measure just a few μm (10^{-3} mm) and contain 75–85% of water, have since become ubiquitous.

Wherever moisture and nutrients are available they reproduce explosively–exponentially, that is. Thus

$$m_1 = m_0 \cdot e^{\mu t}$$

where

m_1 = number of microbes/ml at point of time t ,

m_0 = the number at point of time 0,

μ = specific growth rate.

A gram of fertile arable soil contains some 2–5 thousand million microbes. Waste water–and likewise, indeed, fluids used for technical purposes (e.g. lubricoolants)–contain between 1 and 10 million microbes per millilitre, while even high-quality drinking water has up to 100 microbes per millilitre.

All organic matter existing on earth is decomposable by micro-organisms, which use it as their source of nutrition. Those known as mineralizers are dependent on oxygen as their source of energy. The end-products of decomposition processes in which oxygen is consumed, which are known as mineralization processes, are inorganic compounds and carbon dioxide, which, together with water and light, are used by plants for photosynthesis. The organic matter produced by photosynthesis is the principal nutrient of the great majority of living organisms and the prerequisite of the existence of life.

Where oxygen is absent there are microbe species which obtain their energy from different sources. Methane bacteria get it by reducing carbon dioxide to methane and are thus responsible for the formation of marsh gas or natural gas. Sulphate-reducing bacteria obtain their energy by reducing sulphate to hydrogen sulphide under unaerobic conditions, causing many objectionable odours.

As in alcoholic fermentation, the production of sauerkraut, the ripening of cheese, the baking of bread, the production of penicillin, the purification of waste water at biological treatment plants and the production of bio-gas from agricultural waste micro-organisms may be extremely useful, and even indispensable, to Man. Yet in other cases they are harmful or highly dangerous: by causing infectious diseases, by forming poisonous or carcinogenic metabolites and wherever–in addition to decomposing wastes–they attack valuable materials, disturb production processes or impair the quality of products. According to estimates, microbial decomposition destroys materials to the value of at least one hundred thousand million dollars several times over every year.

It was not until the 19th century that the causes of fermentation, decay and infectious diseases were investigated and the way opened to measures directed specifically against the harmful micro-organisms.

Pasteur, the pioneer in this field, whose discoveries were made possible by Leeuwenhoek's invention of the microscope in the 17th century, initiated the war against harmful micro-organisms with a physical method: he killed them with heat. Chemical methods—the use of substances that destroy or inhibit microbes—soon followed. In 1867 Lister made use for the first time of the antiseptic properties of 'carbolic acid' (phenol) to kill bacteria on medical instruments, dressings and wounds. In 1868 Brown and Frazer postulated in the Transactions of the Royal Society of Edinburgh: "There can be no reasonable doubt that a relation exists between the physiological action of a substance and its chemical composition and constitution, understanding by the latter term the mutual relations of the atoms in the substance". Consequently they set up the equation that changes in physiological/biological activity of a substance (ΔA) are a function (f) of changes in chemical structure (ΔC): $\Delta A = f(\Delta C)$.

Anyone using chemical methods to combat material destruction by microbes, has more than 250 commercially available microbicides to choose from, not to mention numerous formulations of these active ingredients. Microbicides and formulations thereof differ considerably in their physical and chemical properties, effectiveness and spectrum of activity. This diversity is the natural consequence of the variety of materials of widely differing physical and chemical properties and uses that have to be preserved. The properties of the microbicides clearly have to be adapted to the properties of the materials they are to protect. In addition there are many different types of microbes capable of attacking materials. Bacteria are often divided into those which are stainable with iodine/crystal violet (Gram-positive bacteria) and those that cannot be stained in this way (Gram-negative bacteria). It is important to know that the differences in stainability arise from differences in the morphology and composition of the bacterial cell wall, which surrounds and protects the cytoplasm and the cytoplasmic membrane; see Figure 1 in chapter 2. By virtue of its composition and structure, the cell wall, unlike the cytoplasmic membrane, is able to keep the shape of the bacterial cell unchanged, i.e. it withstands the high osmotic pressure exerted by the cytoplasm within the cell. The fundamental difference between Gram-positive and Gram-negative bacteria is that the latter have a so-called outer membrane, which is not found in Gram-positive bacteria. The different barriers differ in their capability to prevent the penetration of microbicides, which explains the variation in sensitivity of different types of microbes towards one and the same microbicide.

It is wishful thinking to imagine that the biodeterioration of materials can be fully prevented with only a handful of different microbicides. Also proceeding from the assumption that in dependence of different targets on and in the microbial cell one has to make corresponding changes in the chemical constitution of active agents, this does obviously not lead to reduction of the number of microbicides to inhibit biodeterioration. On the contrary, growing advances in understanding mechanisms of biodegradation could contribute to the development of microbicides that interfere with a specific biodegradation mechanism. However, different mechanisms then would require different inhibitors; in consideration of the extensive and expensive registration procedures for new microbicides this is a severe problem not evident in not highly target-specific broad-spectrum biocides or microbicides (Green, 2000).

In the light of the knowledge that the earth's raw material resources are limited, the need for the development of modern microbicides which protect valuable products from loss of quality and deterioration is more urgent than ever. They not only protect a great number of perishable products from depreciation and destruction, but also prevent problems in industrial processes caused by algae, formation of slime and biofilms, and microbial induced corrosion.

The demand for microbicides by chemical-technical industries led at first to the use of such oxidizing agents as chlorine and hydrogen peroxide, which, though intrinsically very effective, are unstable. Other chemicals followed, but the requirements that had to be met were still modest, comprising little more—in the case of microbicides used to protect materials—than the need:

- to have a strong microbicidal effect and broad spectrum of activity;
- for the greatest possible stability; persistence;
- for economy in use.

This short list, still remarkable for its simplicity, led inevitably to the organometallic compounds and polyhalogenated phenol derivatives—active ingredients which are both highly effective and highly persistent and which may indeed be termed 'biocides', since they are harmful not only to micro-organisms, but also to plants, insects, snails, fish and other organisms—in short, to all forms of life. Pressure for replacement of such biocides is growing and the end of their use is in sight.

In the meantime, however, a new catalogue of requirements has evolved—or, rather, the earlier ones have been modified to meet the needs of present-day civilization as seen in its heightened sense of environmental responsibility and desire for quality of life.

The characteristics demanded of microbicides for the protection of materials today are as follows:

- the possession of degrees of stability and spectra of activity graduated according to the applications;
- very low toxicity;

- very low ecotoxicity;
- economic in use.

The user no longer insists on persistence at any price, but is content with limited persistence graduated to the application: for stability or instability as dictated by the circumstances.

Often furthermore, there is no need to use a microbicide which, like pentachlorophenol, combines fungicidal, bactericidal, algicidal and insecticidal effects, since a satisfactory result can be obtained with a product that controls only one kind of organism. If this product is also effective at very low concentrations, so much the better. In other words, microbicides must now be tailored to the intended use and to the microbe species to be controlled. But obstacles to the development of new and 'progressive' microbicides for material protection, that arise from complex and labour-intensive bureaucratic registration procedures, are delaying desirable changes. One result of the consequent disincentive to innovation is that manufacturers are seeking to broaden the application of those progressive microbicides that have already overcome these hindrances. For this purpose they are either developing additional formulations or combinations of individual microbicides with extended activity spectra, or exploiting synergisms that enable ingredients to be used at lower concentrations.

One should not overlook the fact, however, that the use of microbicides for material protection represents a significant contribution to environmental protection.

Let us see what this means in practice. Well preserved functional fluids—metalworking fluids, for instance—can be used longer than unpreserved fluids and therefore appear less frequently as wastes that are difficult to dispose of and place a burden on the environment. Greater durability of products arising from the use of microbicides also means that replacements are needed less often, with the result that the burdens imposed on the environment by industry may be reduced and that valuable raw materials of limited availability may be spared. This fact will undoubtedly keep the search for new microbicides going. If antimicrobial compounds that are effective at extremely low concentrations can be found, their discovery and development will be justified even if they are comparatively costly. Finally, growing knowledge of the relationship between antimicrobial effect and chemical composition and structure, and—in this context—the opportunities for computer-assisted tailoring of microbicides, will keep the cost of development, which could otherwise become prohibitive, within reasonable limits.

The number of fields of application for microbicides is nearly as huge as the number of microbicides. They reach from fruits to fuel and from toothpastes to drilling muds. The selection of a suitable microbicide will depend first on the type of microbes to be controlled and secondly on the environment in which the active ingredient is to be used. There are available bactericides, fungicides, algicides or active ingredients which are especially effective against yeasts or those having a particularly broad spectrum of activity. The composition or the pH of a material to be protected can already provide some information on the type of microbe attack which may be expected.

Products containing sugars, e.g. lignosulphonates, are mainly attacked by yeasts. Mould-producing fungi prefer substrates with a weakly acid or neutral reaction: they like to grow at air-liquid interfaces, on surfaces, and in aerated products. Bacteria mainly decompose products containing protein and with a neutral to weakly alkaline reaction. In so far as acidification is possible, e.g. to pH 4, this in itself limits the growth of bacteria and aids preservation. Microbial slimes and biofilms build up in process water, and if the water is exposed to light abundant growth of algae also occurs. Microbial growth on surfaces will occur only when sufficient water (relative humidity) is available.

A relative humidity of 80% is conducive to growth, 60–80% may or may not be depending on other factors, but below 60% no growth will occur. For microbial growth on surfaces it is not obligatory that the entire substrate is a nutrient for micro-organisms; it suffices when one component of the substrate, e.g. a plasticizer, can serve as a nutrient or a thin layer of dirt or organic matter which has accumulated on the surface. Although water is essential for microbial growth, microbes can grow even in hydrocarbon fuels which contain only traces of water. This becomes possible if some condensation of water occurs as the temperature decreases; then both bacteria and fungi are capable of growing at the oil-water interface and of producing slimes which may plug filters and lines.

Without regard to details one can distinguish between four general groups of fields of application for microbicides:

1. Industrial aqueous process fluids, e.g. cooling waters, pulp and papermill process waters and suspensions, secondary oil recovery systems, spinning fluids, metal working fluids.
2. In-tank/in-can protection of aqueous functional fluids, e.g. polymer emulsions, water based paints and adhesives, glues, starch slurries, thickener solutions, gelatine, wax emulsions, inks, polishes, pigment and mineral slurries, rubber latexes, concrete additives, drilling muds, aqueous cosmetic and pharmaceutical formulations.
3. Antimicrobial treatment of materials that finally contain little or no water in a free state, e.g. paint and adhesive films, textiles, paper, paperboard, plastics hoses, cords, rubber products, leather, wood.
4. Disinfection of inanimate surfaces and equipment.

It is self evident that in the first two groups of fields of application one needs microbicides of sufficient water solubility, and as far as two-phase systems are concerned, e.g. emulsions, microbicides with a distribution coefficient or partition coefficient which favours the enrichment of the active ingredient in the water phase. For the oil-water partition coefficient K_w^o the following equation of Bean et al. (1965) is valid:

$$C_w = C \frac{\Phi + 1}{K_w^o \Phi + 1}$$

C_w = concentration of microbicide in water

C = total microbicide concentration

Φ = oil/water ratio

In Figure 1 is demonstrated how different microbicides behave differently in an emulsion paint according to their varying partition coefficients.

To fulfil the requirements for the third group of applications it is essential to dispose of microbicides which are virtually insoluble in water and are not volatile, do not cause coloration and are practically odourless. Active ingredients for disinfectants should exhibit microbicidal action within minutes and additionally should have properties which make it possible to prepare liquid, water-dilutable concentrates of the actives.

There are some rules to which one should pay attention in connection with the application of microbicides for the protection of materials.

Perfect starting materials and hygienic practices may not be a substitute for preservatives and disinfectants, but they are essential conditions for their economic use.

The efficacy of a preservative and the concentration level of a microbicide to be added are very much dependent on the germ content of the material to be protected. This is in particular valid for electrophilically active microbicides which in general react irreversibly with nucleophilic components of the microbial cell, that means that they are used up by being effective. But membrane active microbicides which adsorptively coat microbial cell walls are also withdrawn from action at least temporarily if large numbers of microbial cells are present. The exponential growth profile of microbes (p. 1) always has to be taken into consideration.

Damage already inflicted by microbes cannot be undone even by very large additions of microbicides. Since the concentrations of active ingredients normally required for microbe control in general are not sufficient to inactivate exogenic enzymes and thus to prevent enzymatic degradation processes, steps should be taken very

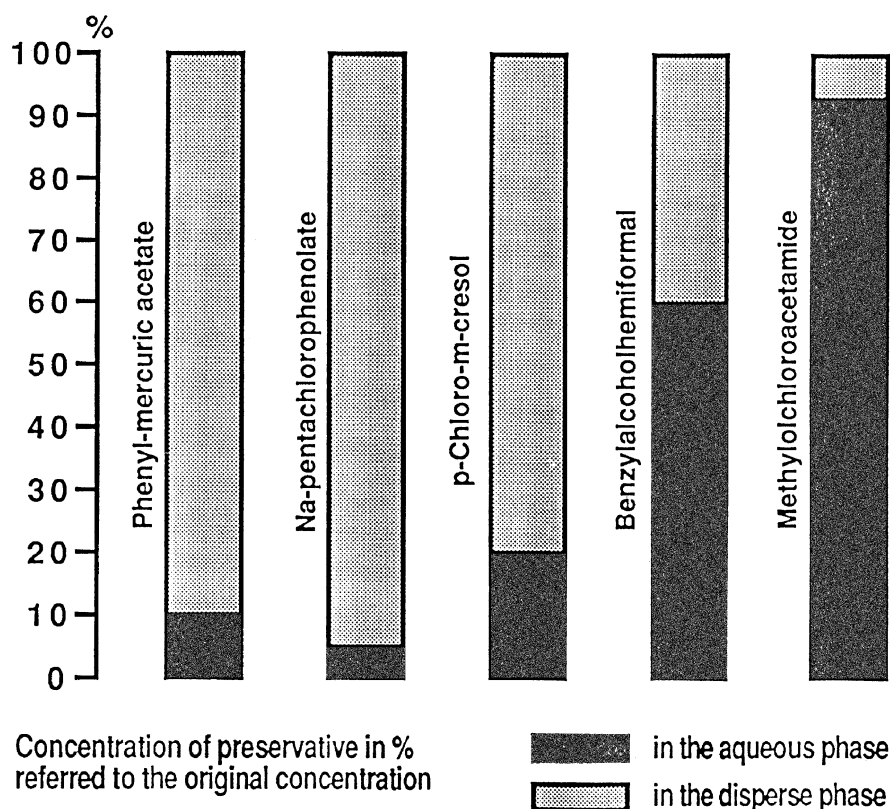


Figure 1 Distribution of various microbicides between aqueous and organic phase of an aqueous acrylic paint formulation.

early to preserve the material, ensuring that enzyme production by microbes does not take place. Oxidizing agents (II, 21.)* and organomercury compounds (II, 19.) are examples of active ingredients being able to inhibit enzymes, e.g. cellulosic material degrading cellulases.

The antimicrobial effect of formaldehyde and formaldehyde releasing agents (II.3.) may be reduced by ammonium ions (formation of hexamethylene tetramine (II, 3.3.1.)).

Phenolic compounds (II, 7.) can irreversibly react with formaldehyde especially at pH value above 9; the reaction products are much lower in activity than the starting products.

Non-ionic surfactants have an adverse effect on the activity of all phenolic microbicides (II, 7.) including p-hydroxybenzoates (II, 8.1.11.); anionic surfactants have considerably less effect. Cationic active ingredients (II, 18.1.-2.) are inactivated by anionic components.

Selecting an appropriate microbicide one has always to take into consideration the pH of the medium to be protected. Degradation/hydrolysis of the microbicide may occur at certain pH values.

There are microbicides which are effective only within certain pH ranges (optimum pH) e.g. glutaraldehyde (II, 2.5.), acids (II, 8.), phenolics (II, 7.), quaternary ammonium compounds (II, 18.1.).

Storage of finished or intermediate aqueous functional fluids at varying temperatures will lead to condensation of preservative-free water in the head space of the storage tank unless a volatile microbicide is used. The condensate will then rapidly become contaminated from its environment. Dripping back on to the surface of the storage product a layer of preservative free condensate forms which is highly susceptible to microbial growth. It is therefore advisable to use microbicides which exhibit head space activity or to store the protected aqueous fluids at more or less constant temperatures.

If one is confronted with the task of solving a biodeterioration problem, one can expediently proceed stepwise:

1. Precise identification of the biodeterioration problem.
2. Specification of requirements for an appropriate microbicide.
3. Selection of corresponding microbicides.
4. Pretrials-evaluation.
5. Application of a suitable microbicide under practical conditions.
6. Solution to the biodeterioration problem.

1.2 Evaluation of preservatives

The preservation effects of microbicides in aqueous functional fluids can be ascertained, and reliably effective concentrations established, by a method which, though referred to as classical, has been extensively modified over the years. The basis of the method, which has not itself been changed, comprises three steps:

1. Inoculation of the test medium with micro-organisms.
2. Incubation of the contaminated test medium.
3. Viable cell count or determination of the cell count (cfu = colony forming units) per g or ml.

Each of these steps, however, can be modified in many ways. The test medium can be inoculated with a single, several or numerous micro-organism species. For this one can use pure cultures obtained in the laboratory under optimal conditions of temperature, pH value, humidity, nutrient availability and osmotic pressure, or what are known as acclimated micro-organisms, which are isolated from aqueous functional fluids that have been spoiled by the microbes concerned. Acclimated micro-organisms should be used whenever possible.

Non-acclimated micro-organisms have in many cases died after being transferred from their optimal environment to the test medium, even where this is a blank sample and where such a sample is very susceptible to microbial growth. The lethal stress suffered by non-acclimated test organisms in such cases is caused by the sudden change from one medium to a different and contrasting medium (Cooke et al., 1991). If acclimated micro-organisms are not obtainable and a selection of pure cultures is available, one should always include *Pseudomonas aeruginosa* among the organisms tested because it is a troublesome, difficult-to-control organism that is found very often in fluids that have been spoiled by microbes. According to Kempson (1976) it is safe to assume that a treatment which controls *Pseudomonas aeruginosa* reliably will also be effective against other, less resistant species. It is advisable to repeat the inoculation once, or preferably several times, after a definite incubation time. This shows, without a chemical analysis being needed, whether or not the preservative has been inactivated by the test medium—possibly through hydrolysis or because the preservative has reacted with constituents of the test medium. Such reactions can be accelerated with heat, e.g. by maintaining the temperature at 50 °C for 1 week. Inoculation times can be varied, just as incubation temperatures can (e.g. 48 h, 27 °C). As an alternative to a viable cell count, a streak assessment can be done. For this a given amount of the fluid to be

*see Part Two – Microbicide Data

investigated is spread on a sterile nutrient substrate in a petri dish, after which the intensity of the microbe growth arising from the streak is evaluated.

Evaluating antimicrobial treatments of other materials is less easy than checking the preservation of aqueous functional fluids. Help is available, however, from institutes and microbicide suppliers that routinely use a number of standard methods for specific materials, such as plastics, wood, paint films and textiles. The same applies to the testing of disinfectants. The final test, however, is one under practical conditions, which may vary very much according to the place and region and which cannot always be simulated in the laboratory.

If microbicides for the protection of materials are to be used appropriately and without risk to humans and the environment, the user, too, should be as familiar as possible with their property patterns, aware of the toxicological and chemicophysical properties of the active ingredients and, lastly, know the concentrations at which they are effective under practical conditions. It is hoped that the present book will contribute to this goal.

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2 Relationship between chemical structure and activity or mode of action of microbicides

W. PAULUS

2.1 Introduction

Microbicides for preventing the biodeterioration of materials and thus preserve their value as long as possible have been developed formerly more often empirically, however, today more often by design, because of greater knowledge and understanding of relationships between chemical structure and activity and mode of action of microbicides (Franklin and Snow, 1989).

Within groups of chemically related substances it is possible to correlate changes in biological activity with variations in chemical structure respectively in structural elements, or variations in chemical and physical properties. The chemical biology of the microbial cell is extremely complex, however. The chemist stands opposite large molecular systems and tries to solve the problem by assuming that only a small bounded part of a system has structure which interacts with structural (toxophoric) elements of microbicides. The resort to such abstraction allows to predict antimicrobial effectiveness of substances bearing toxophoric groups able for instance to react with amino, amido, thiol groups, as these are part of large molecular systems such as proteins and nucleic acids. Corresponding examples are aldehydes or aldehyde releasing compounds, or substances containing an activated N-S bond, on condition that the active ingredients dispose of an adequate molecular size, lipid and water solubility and a minimum of stability under physiological conditions.

Paul Ehrlich (Nobel-prize-winner 1908 for his research in the field of immunology) postulated in 1899: "Corpora non agunt, nisi fixata". According to his opinion the basic requirement for antimicrobial action is the ability of an active substance to associate with the cells. This is the first step of an antimicrobial effect which nowadays in total is defined as the interaction of an active ingredient with specific target sites on and, after transition, in the microbial cell. Accordingly it is a reasonable assumption, that there exists a relationship between the chemical structure of microbicides and their activity or mode of action. However, also structure-related are physical and chemical properties which enable the active ingredient to reach the target regions (Figure 1.). Molecules with a molecular weight higher than 600 in general are not able to penetrate the cell wall. For the passage through the water filled porin channels of the outer membrane of Gram-negative cells microbicides must display hydrophilic properties. The waxy cell wall of Gram-positive mycobacteria is an example of a hydrophobic barrier to hydrophilic active ingredients. Hydrophobic microbicides must be equipped with optimal hydrophilic properties for penetration (Denyer, 1995). Also important for workable microbicides are structure-related characteristics which enable them to be stable and to partition in the phase where their activity is required. In some fields of

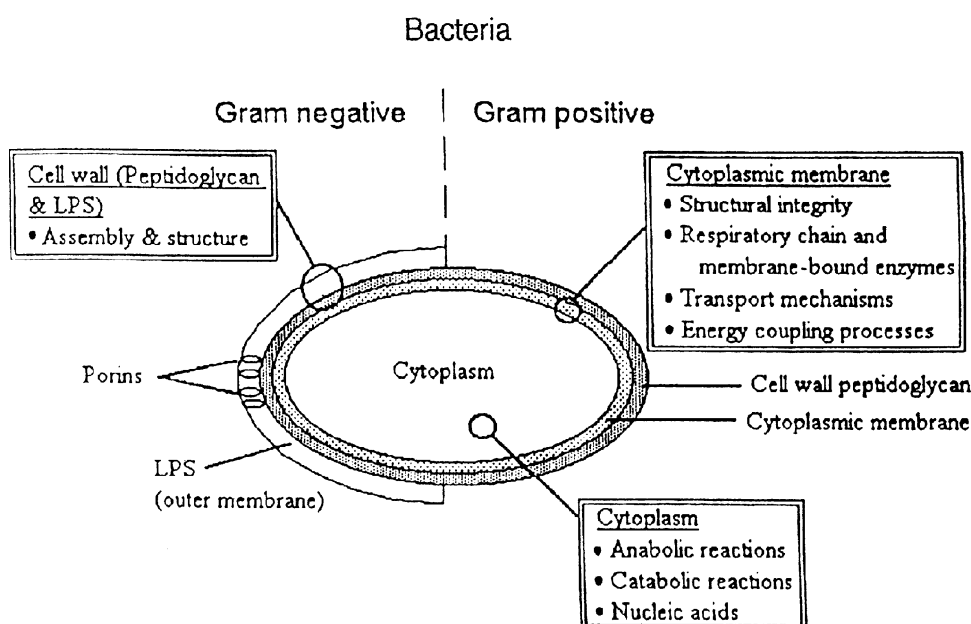


Figure 1 Potential targets for biocides (Denyer, 1995).

application one needs microbicides of high water solubility; in others, active ingredients of minimum water solubility, but solubility in non-polar solvents and low volatility, are required.

2.2 Classification of microbicides

A large number of microbicides for material protection can be characterized according to their mechanism of action as either electrophilically active, membrane-active, or able to form chelates. This subdivision is indeed a rough one, but nonetheless helpful, although it is not always possible to classify active ingredients according to this distinction completely. The first step in the interaction of microbicides with microorganisms can be described as a physical process which occurs between the cell surface and the active ingredient. As much of the chemistry and functional physiology of the microbe cell, including energy production, protein synthesis, and nutrient adsorption takes place at the cell membrane, microbicides on their way to the membranes come across a lot of sites where they can exhibit their antimicrobial efficacy. Subsequent to association then follows as a second step, transport across the cell membranes, if the chemical and physical properties of the active ingredients allow this. According to Bergethon (1998) such a transport can be classified as either passive or facilitated. Passive transport is the diffusion of a component down a chemical potential gradient. The rate of flux through the membrane will be related to the movement of the compound from the bulk solution onto the surface of the membrane. Then the movement through the membrane will depend on the diffusion coefficient for that compound in the membrane milieu. Finally there must be a desorption of the compound from the membrane into the other bulk phase that was separated by the membrane. Facilitated transport is needed for molecules whose solubility in the lipid phase is very low. Accordingly transport of these molecules will be very slow. Their movement across the lipid barrier can be facilitated, if they are carried by an amphipathic molecule, or if their lipid solubility is increased by chelation, e.g. transformation of 8-hydroxyquinoline to copper-8-hydroxyquinoline [II, 13.3a.].*

2.2.1 Membrane-active microbicides

Membrane-active microbicides include **alcohols – phenols – acids- salicylanilides – carbanilides – dibenzamidines – biguanides – quaternary ammonium salts** and other active ingredients with cationic character, e.g. **azole fungicides** which also act as chelate formers. As many of the antimicrobial agents which are able to complex metal cations display membrane-activity, they will be considered here as membrane-active microbicides.

Membrane-active microbicides at first act more or less unspecifically by coating the cell wall of the microbe adsorptively (association), a process which is initially reversible for example by dilution, particularly when the agents are added in non lethal concentrations and redressed quickly enough. The adsorption process causes changes in the outer membrane and along the cell wall. These outer barriers eventually lose their integrity, with the result that the microbicidal molecules are allowed access to the cytoplasmic membrane so that they can release their lethal effects: disarrangements in the semi-permeable properties of the cytoplasmic membrane, inhibition of enzymes localized there, escape of essential components from the cytoplasm, precipitation in the cytoplasm and finally desintegration of the cells.

Example 1. Acids [II, 8.] and phenol derivatives [II, 7.].

The synonym for phenol itself “carbolic acid” indicates that phenol and its derivatives are acidic, i.e. like acids they dissociate hydrogen ions and are able to form salts ($\text{Ph-OH} \rightleftharpoons \text{Ph-O}^- + \text{H}^+$). In the same way as acids, phenol derivatives release their antimicrobial effect in an undissociated state, since in this state only they are membrane-active and exhibit affinity to the negatively charged surface of the microbial cell, whereas a negatively charged anion is repelled. For this reason one has to bear in mind the pK_a values (see Table 1.) of the different acids and phenol derivatives in order to take optimal advantage of their antimicrobial power during application. The pK_a value is the pH at which 50% of an acid or phenol is in the dissociated state. According to the pK_a values listed in Table 1. acids such as **sorbic acid** [II, 8.1.5.] or **benzoic acid** [II, 8.1.9.] may be used effectively as preservatives in acidic media only (pH values up to 4.5), whereas **parahydroxybenzoates** [II, 8.1.11.] are also active in slightly alkaline media of pH values up to 8.5; however, certain microbicidal phenol derivatives exhibit their antimicrobial effectiveness even at pH values up to 10–11.

The antimicrobial activity of the phenol molecule can be influenced in various ways by introducing different substituents into the phenyl nucleus. Alkylation of phenol produces derivatives with reduced acidity, higher surface-activity and lower water-solubility, but better lipid-solubility. Overall these changes in properties mean an increased antimicrobial efficacy. This reaches a maximum with the unbranched C_6 -chain in the para-position. Branched chains with the same number of C-atoms do not increase the effectiveness of the corresponding phenol derivatives to such an extent. The introduction of alkyl chains with more than six C-atoms does not lead to a

*see Part Two – Microbicide data

Table 1 pK_a values of acidic compounds used as antimicrobials.

Acidic compound	pK _a
Formic acid	3, 8
Acetic acid	4, 8
Propionic acid	4, 9
Lactic acid	3, 8
Dehydroacetic acid	5, 4
Sorbic acid	4, 8
Benzoic acid	4, 2
Salicylic acid	3, 0
Methyl-p-hydroxybenzoate	8, 5
Propyl-p-hydroxybenzoate	8, 1
3-Methyl-6-isopropylphenol	10, 6
2-Phenylphenol	11, 6
2-Benzylphenol	11, 6
2-Benzyl-4-chlorophenol	11, 0
3-Methyl-4-chlorophenol	10, 1
2,2'-Dihydroxy-5, 5'-dichloro-diphenylmethane	8, 7/12, 6
2,4,6-Trichlorophenol	8, 5
Boric acid	9, 1

further rise in efficacy; this is due to the decreasing water-solubility. Halogenation of phenols causes the generation of phenol derivatives, the acidity and antimicrobial effect of which increase with the number of halogen atoms introduced. The phenol derivatives most used today as microbicides besides **ortho-phenylphenol (OPP)** [II, 7.4.1] are alkyl phenols which contain a chlorine atom in the para-position, such as **p-chloro-m-cresol (PCMC)** [II, 7.3.1.], **p-chloro-m-xenol (PCMX)** [II, 7.3.2.] and **p-chloro-o-benzylphenol (CBP)** [II, 7.3.5.].

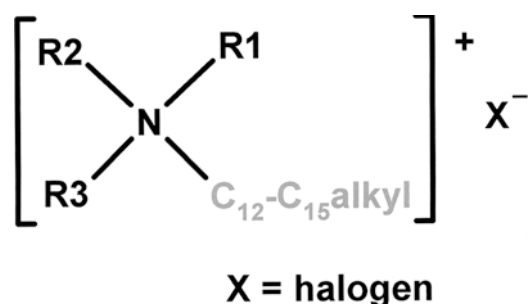
In view of the modes of action mentioned above it is not surprising that microbicidal phenol derivatives have a broad spectrum of activity covering bacteria, yeasts and fungi. It is also logical that Gram-negative bacteria are more resistant to many phenolic compounds than Gram-positive bacteria since they have an outer membrane. In the case of PCMC, the water-solubility, lipid-solubility, acidity and molecular size are such that the microbicide is also considerably effective against Gram-negative pseudomonads. PCMC's well balanced spectrum of effectiveness is demonstrated by the minimum inhibition concentrations listed under II, 7. in comparison to those of PCMX and CBP.

Example 2. Cationic surface-active microbicides [II, 18.]

Surface-active microbicides with cationic properties include not only the widely used **quaternary ammonium compounds (QACs)** but also **long-chain (C12-C15) aliphatic alkyl amines, aliphatic diamines, guanidines and biguanides** (Paulus, 1993a; Woodcock, 1988). The cationic surface-active microbicides are attracted by the negatively charged surface of the microbial cell and particularly strongly adsorbed by ionic interaction with phospholipids of the cell wall. The cell wall thereby loses its function as a protective barrier so that the active ingredients are able to penetrate to the cytoplasmic membrane. The cationic microbicides impair permeability until the cytoplasmic membrane no longer functions as a semi-permeable membrane (Denyer, 1995; Wölfel et al., 1985).

The antimicrobial activity of QACs depends on their structure and size (Figure 2.); a long-chain alkyl group consisting of 12 to 16 carbon atoms is essential for their effectiveness. QACs bearing the C₁₄-alkyl chain exhibit a maximum of antimicrobial activity which increases with temperature and pH. At pH 3 QACs are widely ineffective as the negatively charged surface of the cell then is protected from interaction by protonisation.

Gram-negative bacteria with their complex cell wall structure are much more resistant to cationic microbicides such as QACs than are Gram-positive bacteria. It is assumed that the cationic surface-active QACs form clumps

**Figure 2** Surface-active quaternary ammonium compound.

with Gram-negative cells, forming a protective barrier around the intact cells within the conglomerates (Wölfel et al., 1985). However, aralkyl alcohols, in particular 3-phenylpropanol [II, 1.8], can considerably increase the penetration power of QACs and their effectiveness against pseudomonads (Richards and McBride, 1973). See also chapter 5.1. Efficacy of biocides against biofilms.

Example 3. Azole fungicides [II, 14.]

Azole fungicides include both triazole compounds such as **azaconazole**, **propiconazole** and **tebuconazole** (Figure 3) and imidazole compounds such as **imazalil**. They can be characterized as substituted aromatic heterocycles with an unsubstituted nitrogen atom at the meta-position and one nitrogen atom bearing lipophilic groups including a benzene ring (Paulus, 1993b). The antimicrobial action of azole fungicides bases primarily on the inhibition of ergosterol biosynthesis, e.g. in fungi, by blocking the conversion of lanosterol into ergosterol. This occurs through the inhibition of the enzyme C-14 demethylase which normally catalyses the 14- α -demethylation of lanosterol (Kato, 1986). Since ergosterol is an important component of a fungal membrane, the structure and function of the membrane is impaired when ergosterol is not present (Vanden Bossche, 1990). The result is a delay in fungal growth. It has been shown that the presence of an unsubstituted meta-N atom in azole derivatives is essential for their function as inhibitors of sterol biosynthesis (Gadher et al., 1983). Apparently the meta-N atom of the aromatic heterocycles complexes the protohaem iron of cytochrom P-450, a coenzyme of the demethylase. In connection with their lipophilic group, which is structurally variable to a great extent the anti-fungal azole derivatives also display cationic surface-active properties. In this way they are adsorbed by the cell surface, reverse the permeability of the cell membrane at high concentrations and act as fungicides. Finally, the introduction of chlorine atoms to the benzene ring of the azole fungicides is important only so far as this improves the lipid-solubility of the active ingredients.

Example 4. Other chelate formers.

The antimicrobial activity of chelate formers bases partly on their ability to compete for the complexation of metal cations necessary for a functional cell metabolism. However, membrane-activity of the compounds also plays a role. Antimicrobial agents which form chelates include besides the already mentioned azole fungicides **2-mercaptopyridine-N-oxide** (pyrithione) [II, 13.1.3.] (Cooney and Felix, 1972; Chandler and Segel, 1978; Khattar et al., 1988), **8-hydroxyquinoline** (oxine) [II, 13.3.] (Albert et al., 1947; Albert, 1968), and **dithiocarbamates** [II, 11.11.], e.g. zineb, thiram (Ludwig and Thorn, 1960).

Pyrithione is an example demonstrating impressively that for a successful application of the component as a microbicide it is very important to consider the chemical and physical properties of the substance (Figure 4). **Sodium pyrithione** (solubility in water $> 500 \text{ mg litre}^{-1}$) and **zinc pyrithione** (solubility in water 20 mg litre^{-1}) are stable in aqueous solutions between pH 4.5 to 9.5. At pH values < 4.5 the undissociated pyrithione is formed, which in the presence of oxygen or light is very unstable. At pH values > 9.5 the pyrithion salts are oxidized to salts of pyrithione sulfonic acid, the antimicrobial activity of which is negligible. Strong reducing agents attack the N-oxide group of pyrithione: 2-mercaptopyridine is formed which is considerably less effective than the primary product. Oxydizing agents, e.g. peroxides and hypohalites, transform pyrithione initially to **pyrithionedisulfide**,

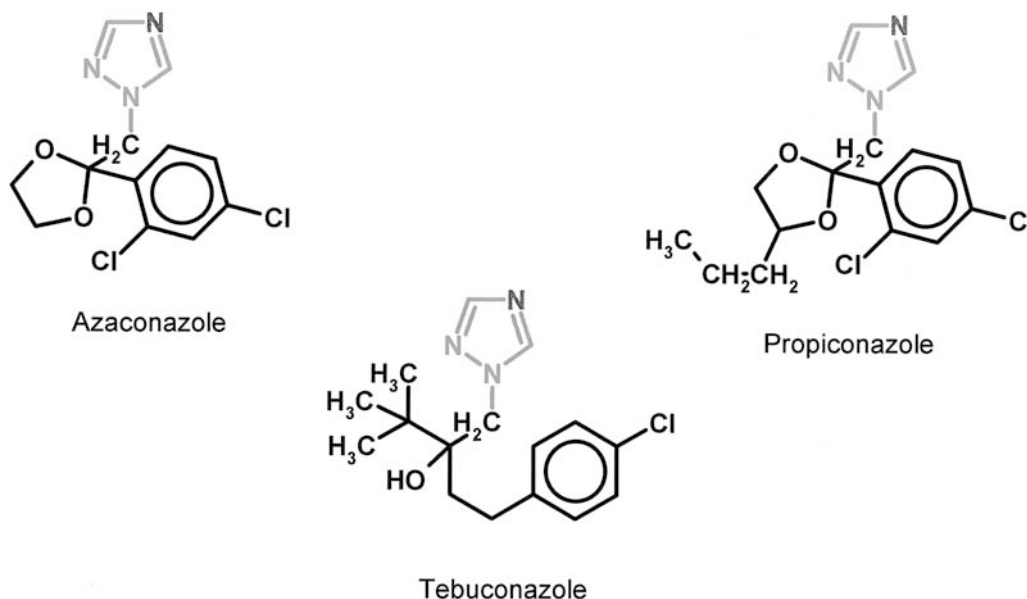


Figure 3 Azole fungicides.

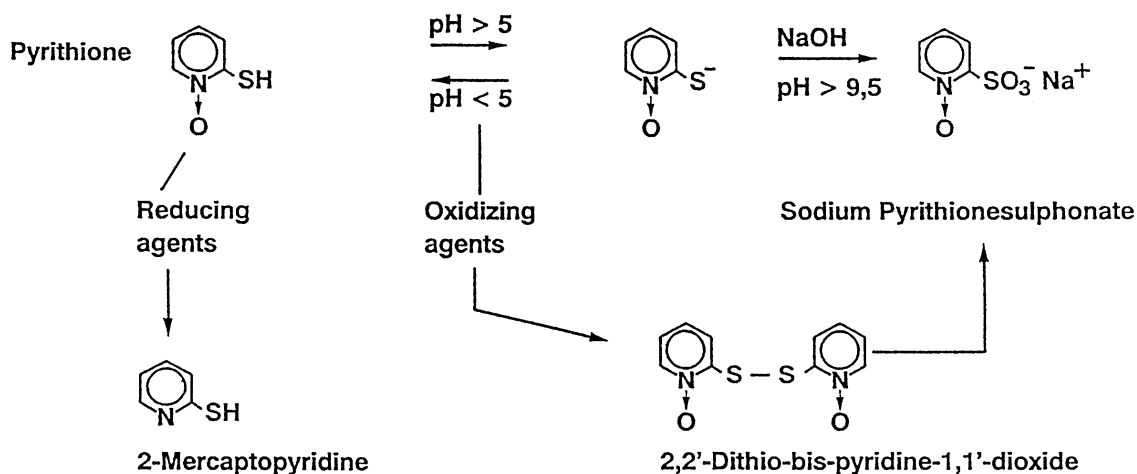


Figure 4 Pyrrhithione's way of acting in different media.

then to pyrrhithionedisulfinate, and finally to the ineffective sulfonate. Pyrrhithionedisulfide is as effective as pyrrhithione and used as a preservative, too. The split of the disulfide bridge with liberation of pyrrhithione occurs preferably in media the pH of which is higher than 7.

Oxine is an example for a microbicide that, because of its low lipid-solubility, needs "facilitated" transport for the movement through the cell membrane. This can be achieved by transforming oxine to the complex comprising oxine and copper: **copper-8-hydroxyquinoline** (Figure 5). The complex possesses considerable lipid-solubility enabling the complexes to pass through the cell membrane and then to dissociate, e.g. into the toxic 8-hydroxyquinoline and the less toxic copper ion. It is notable that oxine copper is indeed around 100 times more effective than oxine itself. The enormous increase in effectiveness is not due to the fact that copper ions pass into the interior of the microbial cell. In general the antimicrobial effect of heavy metal ions, which may also be taken up by non-specific transport systems, is comparatively modest and does not have major practical importance. When microbicidal chelate formers are used in material protection applications, it should be remembered that they also generate complexes with metal cations in the surrounding medium. The complexes with heavy metals, such as copper and iron, are colored and virtually insoluble in water, so that they precipitate. Even traces of the colored complexes may cause intolerable discolorations.

2.2.2 Electrophilically active microbicides

The greatest number of microbicides in use today belong to the group of electrophilically active agents. They include: **aldehydes; compounds bearing a vinyl group in α , β -position to an electronegative group; compounds having an activated halogen atom in α -position and/or vinylogous to an electronegative group; compounds with**

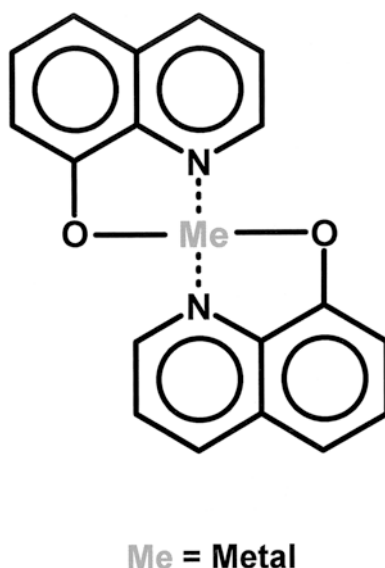


Figure 5 Complexation of a metal cation by 8-hydroxyquinoline.

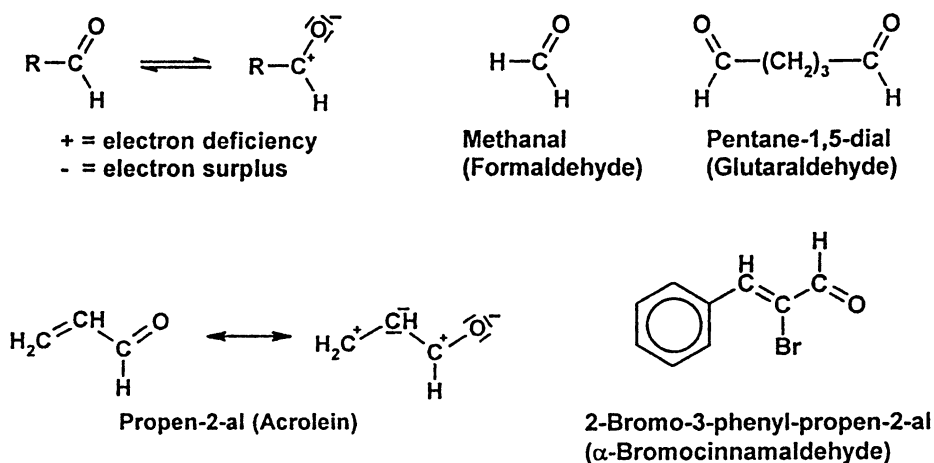


Figure 6 Aldehydes – Different types.

an activated N-S bond as a structural toxophoric element; and organometallic compounds. The antimicrobial effectiveness of these substances results from the fact that they are in search of substrates with a heightened electron density such as nucleophilic components of the microbial cell. A corresponding contact results in an electrophilic addition or substitution (Paulus, 1988). Nucleophilic reaction partners on and in the microbe cell are amino, thiol, and amide groups of amino acids and proteins. As far as these in turn are components of enzymes, the reaction of nucleophilic groups with electrophilically active agents can lead to enzyme inhibition. The antimicrobial efficacy of electrophilically active substances increases with the degree of their electrophilicity; the latter depends essentially on the composition and structure of the substances, i.e. on the electron attracting power of electronegative groups. On their way to target sites beyond the Gram-negative outer cell-membrane and the periplasm and finally beyond the cytoplasmic membrane, electrophilically active microbicides meet a lot of sites to react with. This does not mean in any case partial reduction in activity or even detoxification; an increase in activity is also possible. It all depends on the chemical structure of the active ingredient. Therefore the description of association and transport of microbicides to cells as a physical process is in the case of electrophilically active microbicides not quite correct; it applies preferably to membrane-active microbicides (Diehl and Chapman, 1999). To the advantages offered by electrophilically active microbicides belongs the fact that they are intrinsically non-persistent and do not accumulate in the environment, a property that very often outweighs the disadvantages imposed by their limited stability and duration of activity.

Example 1. Aldehydes [II.2.]

Different types of aldehydes are shown in Figure 6. The electrophilic character of aldehydes is a consequence of the deficit of electrons on the carbonyl atom which enables the aldehydes and aldehyde releasing compounds to react with nucleophilic cell components as is demonstrated in Figure 7.

Among aldehydes **formaldehyde** [II.2.1.] is the compound with the lowest molecular weight (M_r 30) and therefore also the one with the lowest degree of sterical hindrance. The very reactive substance presents the most effective monoaldehyde. Dialdehydes dispose of two toxophoric groups, that means in this case two electrophilic centers able to interact with microbial cell constituents and to undergo manifold connection reactions. **Glutaraldehyde** [II.2.5.] presents the most important dialdehyde used as a microbicide; although not introduced as an antimicrobial substance before 1962, it has been studied and reviewed extensively in the meantime (Gorman et al., 1980; Power and Russel, 1989) and developed into a remarkably active ingredient. It is the most effective

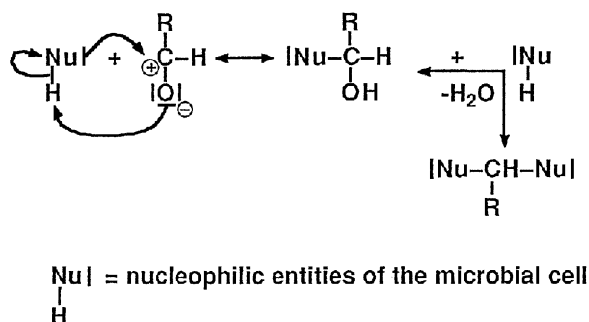


Figure 7 Aldehydes – Mode of action.

dialdehyde offering several structural related advantages in comparison to formaldehyde: Higher activity and rapidity of action; bacteria are killed within one minute; greater ability to penetrate the cell wall; lower irritation potential. As an unsaturated aldehyde **acrolein** [II.2.6.] should be mentioned. It contains a vinyl group in α , β -position to the carbonyl group which results in two electrophilic centers. These impart relatively high reactivity and thus antimicrobial efficacy to the small acrolein molecule. Its effectiveness in very low concentrations covers not only microorganisms but also other organisms such as plants, cold- and warm-blooded animals, so that acrolein can be considered a biocide. Like acrolein, **α -bromocinnamaldehyde (BCA)** [II.2.8.] is an unsaturated aldehyde. However, it contains an activated halogen atom in addition to the activated vinyl group. BCA is nonetheless more stable (less reactive) and therefore less effective and toxic than acrolein, since the phenyl group in BCA ensures a resonance-stabilized system.

Example 2. Substances bearing a vinyl group in α,β -position to an electronegative group.

Microbicides disposing of such a toxophoric structural element – as already mentioned, acrolein and α -bromocinnamaldehyde do that – may add to nucleophilic cell components (Figure 8.) and release their antimicrobial effect in this way. However, such substances are only suitable for practical applications, e.g. as non-persistent slimicides, if the activated vinyl group is part of a resonance-stabilized system (which has been mentioned already in connection with the stability of BCA). Without stabilisation by a resonance system the substances are too reactive and unstable, i.e. polymerisation and reactions with components in the surrounding medium, reactions with ammonia and amines taking precedence (Paulus, 1976).

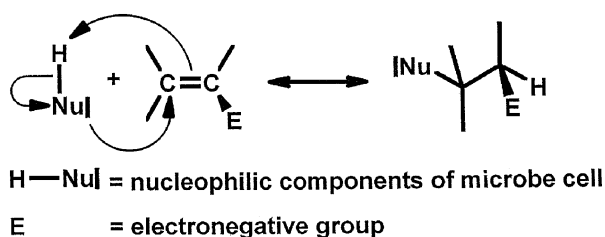


Figure 8 Mode of action of microbicides bearing an activated vinyl group.

However in connection with the application of active vinyl compounds as microbicides one has in any case to regard their reactivity, even if they contain a resonance-stabilized system. **N-(3,4-dichlorophenyl)-N'-acryl-urea** for instance is a reliable fungicide (Paulus et al., 1980) in neutral or acidic media only; in alkaline solutions it reacts intramolecularly to the corresponding dihydrouracil derivative, which is ineffective (Figure 9.). **Aroylethylene** reacts in hydrous ammoniac solution intermolecularly to tris(aroylethyl)amine which precipitates: $3 \text{ Ar-CO-CH=CH}_2 + 1 \text{ NH}_3 \rightarrow \text{N(H}_2\text{C-CH}_2\text{-CO-Ar)}_3$

Here too it becomes obvious that for a successful application of microbicides to prevent biodeterioration problems one always has to bear in mind the chemical and physical properties of the active ingredients.

Polymerisation of the active vinyl compounds during storage can be prevented by adding a so-called "leaving group" (LG); the resulting saturated compounds easily release the effective vinyl derivatives even under

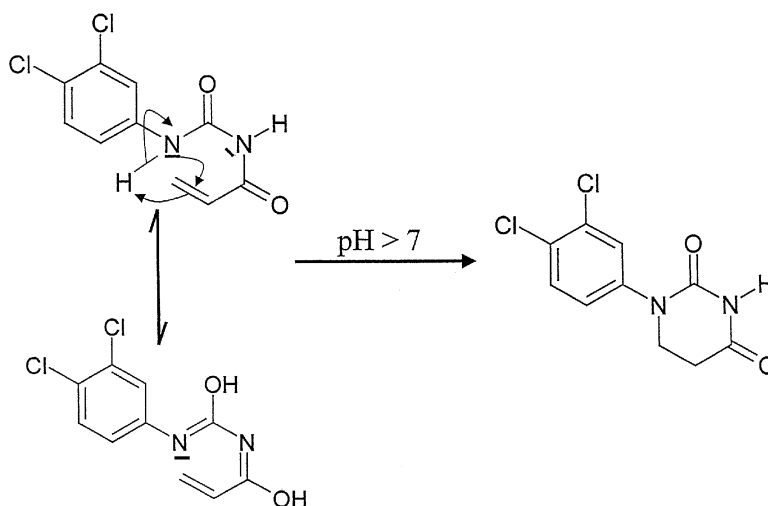


Figure 9 Intramolecular addition reaction of tautomeric forms of N-(3,4-dichlorophenyl)-N'-acryl-urea to N1-(3,4-dichlorophenyl)-5,6-dihydro-uracil.

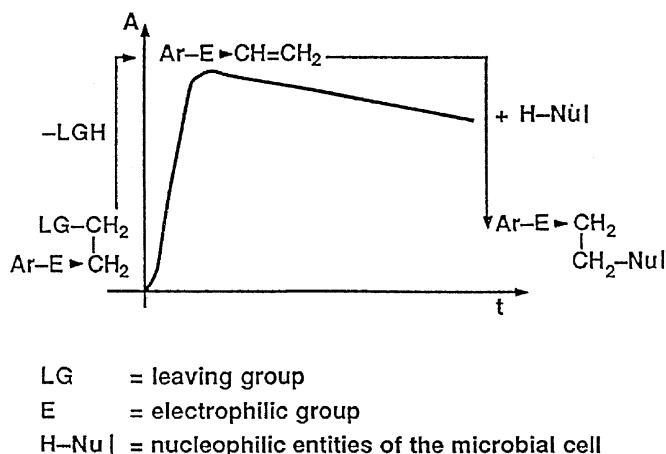


Figure 10 Antimicrobial activity (A) of an aqueous solution of a potential aryl vinyl ketone dependent on time (t) at pH 6.5.

physiological conditions, i.e. room temperature and approximately neutral solutions. In general the rate of cleavage is many times higher than the rates of reactions leading to inactivation of the vinyl compound produced in the cleavage process as illustrated in a biokinetic test (Figure 10.) (Paulus, 1976). This represents an advantage in the practical use of potential vinyl compounds as microbicides.

Example 3. Substances disposing of activated halogen atoms [II, 17.].

The introduction of a halogen atom into the α -position or vinylogously to an electronegative group causes a strong electron deficiency at the respective carbon atom, as the electron affinity of halogen atoms is much higher than that of carbon atoms. The electrophilic carbon atoms are attracted by nucleophilic cell components with hydrogen halide being eliminated. The halovinyl compounds react with nucleophiles in an addition/elimination reaction (Figure 11).

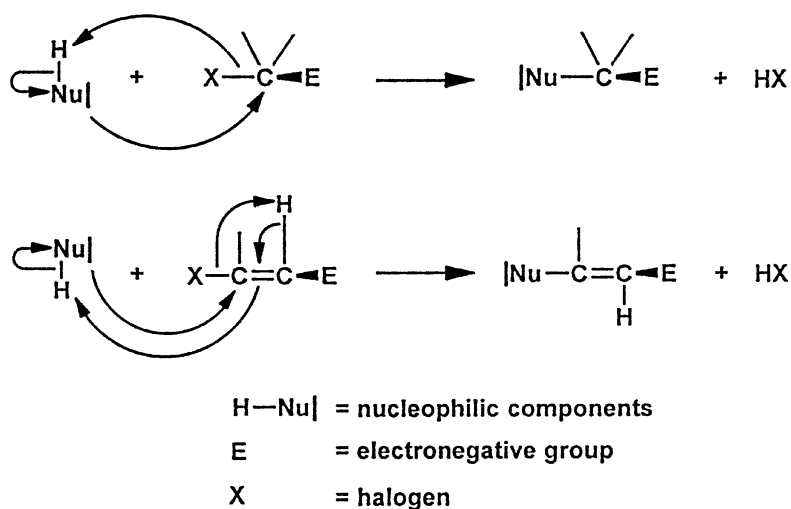


Figure 11 Mode of action of microbicides with activated halogen atoms.

Example 4. Substances containing an activated N-S bond [II, 15. + 16.]

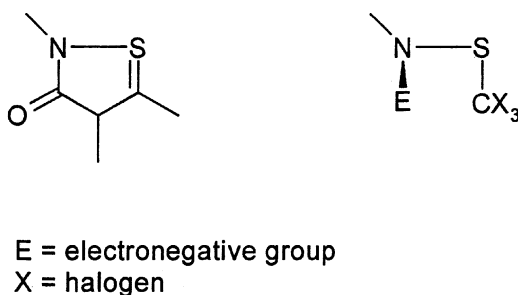
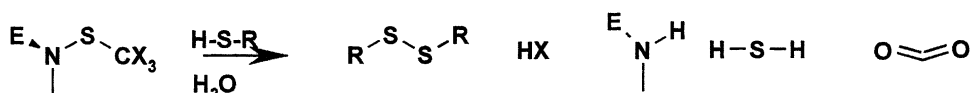


Figure 12 Activated N-S bonds.



E = electronegative group; X = halogen

Figure 13 Reaction of an active N-haloalkylthio compound with a SH group bearing cell component (nucleophile).

Microbicides belonging to this substance class are used on a large scale; they include certain N-haloalkylthio compounds (Lucken, 1966) and isothiazolinone derivatives (Crow and Leonard, 1965; Lewis et al., 1971) [Figure 12.]. In principle there is no difference in the first step in the mechanism of action of N-haloalkylthio compounds and isothiazolinone derivatives with activated N-S bonds; both may react with SH groups found in cell components, with the N-S bond being split off and the formation of disulfides.

For N-haloalkylthio compounds the course of the reaction is summarized in Figure 13. (Owens and Blaak, 1959).

N-haloalkylthio compounds of antimicrobial activity are obtained according to the reaction scheme in Figure 14. by the reaction of haloalkylsulfenylchlorides, e.g. Cl-S-CX_3 ($\text{X} = \text{halogen}$), with $(\text{R}_1\text{R}_2)\text{N-H}$ compounds on condition that the N-H group has acidic character; this is the case, if R_1 and/or R_2 present an electronegative group. The results in Table 2, clearly show that substitution of basic H atoms by the S-CX_3 radical produces inactive substances with N-S bonds of excessive stability. However, the minimum inhibition concentrations (MIC) also clearly demonstrate that electrophilically active substances result if starting materials such as carboxylic acid amides disposing of an H-atom with acidic character are used (Paulus and Kühle, 1986). Apparently there exists a relationship between the antimicrobial activity of N-haloalkylthio compounds and the stability of their individual N-S bonds. This relationship is illustrated in Figure 15. According to this curve N-trihaloalkylthio compounds are especially active when their N-S bond displays a medium level of stability or reactivity (electrophilicity). The N-haloalkylthio compounds not only represent a huge reservoir of microbicides, but also the possibility for the synthesizing chemist to tailor active ingredients which possess the required physical-chemical properties for a specific application. Two compounds shall be mentioned here for demonstration (Figure 14.): **Fluorfolpet** [II, 16.2.], an active ingredient which is thermostable, colorless and odorless, non-volatile, lightstable, practically insoluble in water and slightly soluble in non-polar solvents. Fluorfolpet therefore presents an active ingredient useful for the antimicrobial treatment of plastics. **Tolyfluanid** [II, 16.6.] presents an active ingredient which in contrast to Fluorfolpet is not thermostable, but substantially soluble in non-polar solvents; it is therefore used successfully for the fungicidal and algacidal treatment of solvent based paints including antifouling coatings and non-film-forming decorative wood stains.

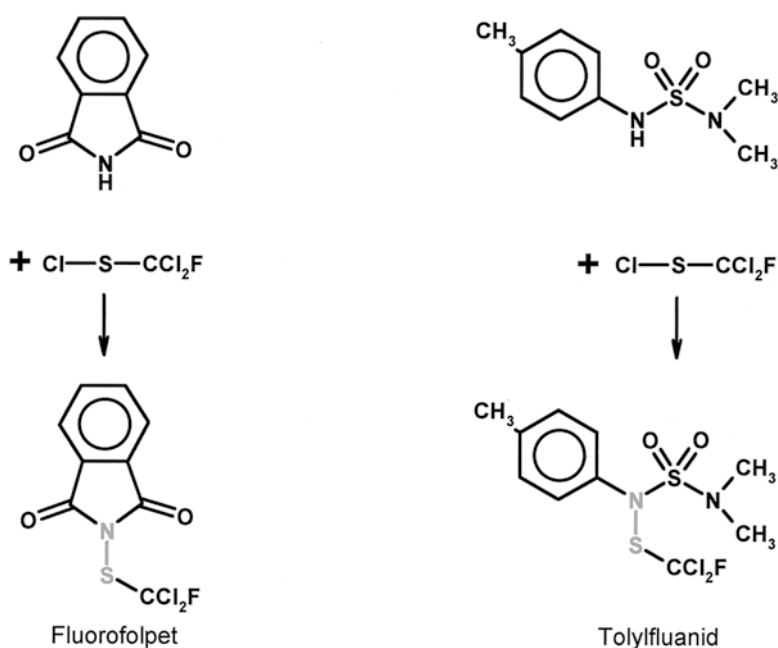
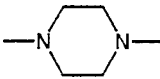
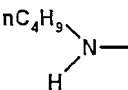
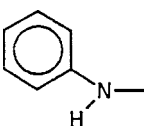
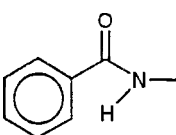
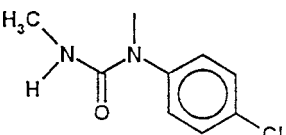
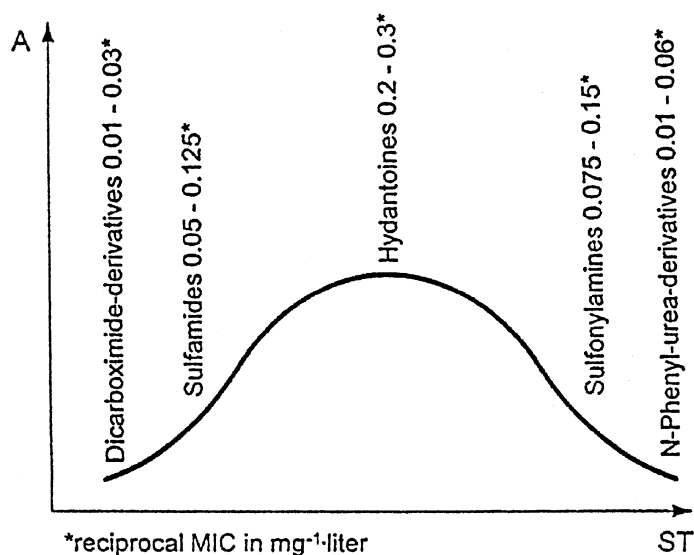


Figure 14 Formation of a N-haloalkylthio compounds containing an activated N-S bond.

Table 2 Antimicrobial activity of R-S-CX₃ compounds produced from substances with H atoms of different reactivity. -Minimum inhibition concentrations (mg. litre⁻¹) in nutrient agar.

R-S-CCl ₂ F group R	Test organisms							
	<i>Alternaria alternata</i>	<i>Aspergillus niger</i>	<i>Auerobasidium pullulans</i>	<i>Chaetomium globosum</i>	<i>Coniophora puteana</i>	<i>Penicillium glaucum</i>	<i>Polyporus versicolor</i>	<i>Trichoderma viride</i>
(nC ₄ H ₉) ₂ N—	>1000	>1000	>1000	1000	100	>1000	>1000	>1000
		>1000		>1000		>1000		
	1000	>1000	500	500	35	>1000	>1000	>1000
	750	>1000	500	350	35	>1000	1000	>1000
		100		100		50		
	15	50	20	20	1	100	35	500

**Figure 15** Antimicrobial activity (A) of N-trihalomethylthio compounds deriving from different acidic N-H compounds versus the stability ST of their N-S bond.

Substances containing an isothiazolone ring, such as **2-methyl-isothiazolin-3-one** (MI), **benzisothiazolin-3-one** (BIT), **2-methyl-4,5-trimethylene-isothiazolin-3-one**, also contain activated N-S bonds which may react with nucleophilic cell entities with opening of the isothiazolone ring, thus exerting antimicrobial activity (Miller et al., 1975).

In the case of MI [II, 15.1.] this mechanism leads to the formation of a 3-dithio-N-methyl-acrylamide (I) which can further react with thiol compounds to generate 3-mercapto-N-methyl-acrylamide (II) and corresponding disulfides of thiol compounds (Collier et al., 1990). A tautomeric resonance-stabilized form of II is

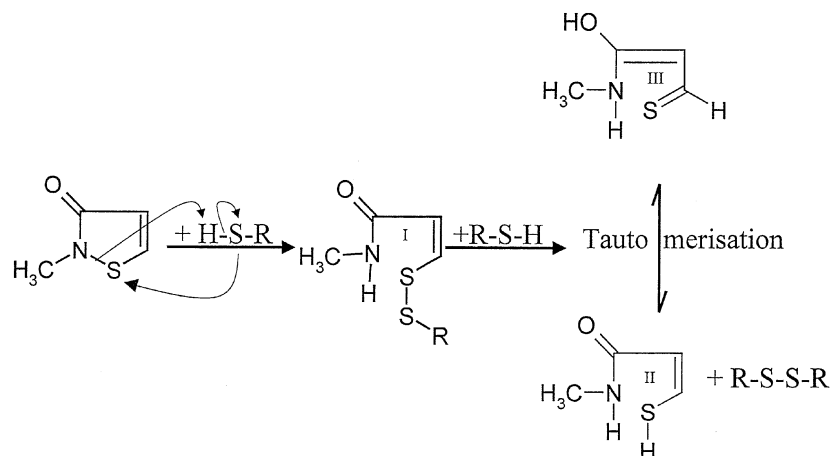


Figure 16 Reaction of 2-methyl-isothiazolin-3-one-with SH-components of the microbial cell.

3-hydroxy-3-methylamino-thioacrolein (III) (Figure 16.), a highly reactive substance (see Example 1. Acrolein), capable of reacting with a great variety of cell components having amino, amide, thiol, and hydroxyl groups, but competitively with corresponding compounds in the surrounding medium.

Isothiazolones containing a chloro atom in 5-position, or two chloro atoms in 4,5-position, such as **-5-chloro-2-methyl-isothiazolin-3-one** (CMI) [II, 15.2.] and **4,5-dichloro-2-(n-octyl)-isothiazolin-3-one-** [II, 15.5.] contain in addition to the activated N-S bond a second toxophoric structural element, namely a chloro atom in the vinyl position, or two chloro atoms in α,β -position to the electronegative carbonyl group (C=O) (see Example 3.). Accordingly their efficacy exceeds that of the non-halogenated isothiazolones significantly (Collier et al., 1990; Diehl and Chapman, 1999). The CMI ring opens as does the MI ring and generates 3-mercapto-3-chloro-N-methyl-acrylamide (IV) which tautomerises (prototropic tautomerisation) to the resonance stabilized 3-hydroxy-3-methylamino-thioacrylchloride (V) (Figure 17.). V is an extremely reactive substance, especially sensitive to hydrolysis, but also able to react readily with cell components. Chloroisothiazolinones can be regarded as transport forms for the described thioacrylchlorides which otherwise one can hardly handle as they hydrolyze to monothiomalonicacid monoamide derivatives. The oral and dermal toxicity of chloroisothiazolones correspond to the described chemical properties of the compounds. The well known fact that the antimicrobial effect of isothiazolones and chloroisothiazolones is strongly antagonised by materials containing SH groups is in line with their mechanisms of action.

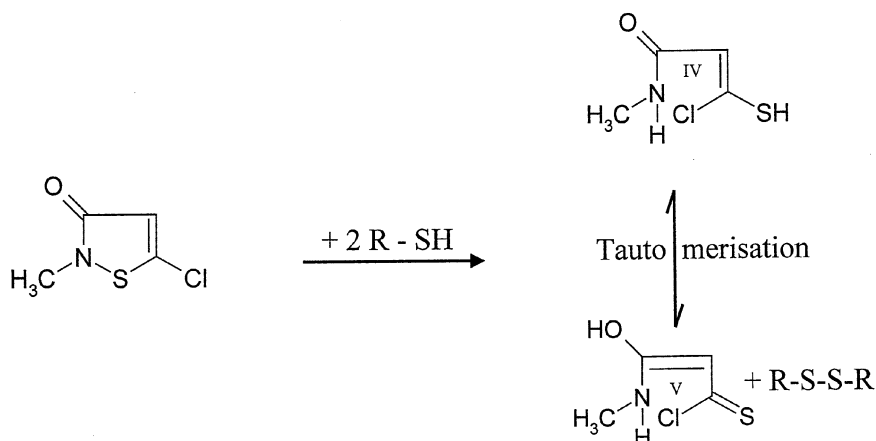


Figure 17 Reaction of 5-chloro-2-methyl-isothiazolin-3-one with SH components of the microbial cell.

Example 5. Organometallic compounds [II, 19.].

Organometallic compounds such as **phenyl mercury acetate** and **tributyltin fluoride** also come into the category of electrophilically active biocides, as they dissociate cationic radicals, e.g. phenyl-Hg⁺, or (butyl)₃Sn⁺, which can react with nucleophilic entities of the microbial cell and thus seriously disturb metabolism (Corbett et al., 1984; Figure 18.). An example is their reaction with essential SH groups, thus causing inhibition of enzymes. This is not restricted to endogenous enzymes, but also affects exogenous enzymes such as cellulases which are not generally inactivated by standard application concentrations of other preservatives.

Table 3 Sensivities of Gram-negative and Gram-positive Bacteria to Phenol Derivatives Arranged in a Succession of Increasing Degrees of Hydrophobicity – Minimum Inhibition Concentrations (mg/l) in Nutrient Agar

Phenol derivative	M_r	Test organisms			
		<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas fluorescens</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
3-methyl-4-chloro-phenol [II, 7.3.1.]	142.5	800	800	250	200
2-phenyl-phenol [II, 7.4.1.]	170.1	1500	1500	200	100
Chlorophen [II, 7.3.5.]	218.5	5000	>5000	3500	20
Dichlorophen [II, 7.7.3.]	269.1	>5000	3500	100	5

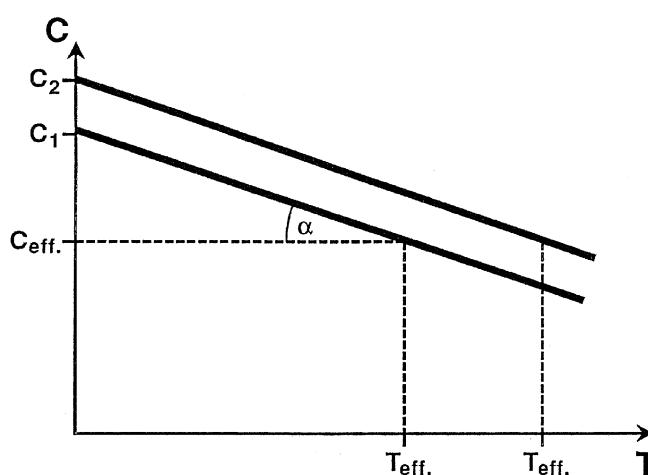
formaldehyde and formaldehyde-releasing compounds is a case of intrinsic resistance attributable to the presence of the constitutive enzyme formaldehyde dismutase in these microbes (Adroer et al., 1990). The stoichiometric dismutation of formaldehyde to methanol and formic acid, known to chemists as Cannizzaro's reaction, occurs under physiological conditions in microbe cells in which formaldehyde dismutase is present. Furthermore, if formaldehyde is supplied, this promotes in the cells the formation of formaldehyde dehydrogenase, an enzyme that catalyses the conversion of formaldehyde to formic acid (Kato et al., 1984).

The formation of biofilms by micro-organisms can be regarded as a physiological (phenotypical) adaptation, and thus represents an intrinsic mechanism of microbial resistance to antimicrobial agents, as biofilms up to 1000 times less susceptible towards a wide variety of different microbicides than are the equivalent planktonic cells (see chapter 5.1.). In biofilms, adhering irreversible to surfaces, cells live in close associations of high densities and are embedded in an organic matrix of biopolymers, the so-called extracellular polymeric substances (EPS), i.e. exopolysaccharide glycocalyx polymers, which are produced by the micro-organisms themselves. Assumptions, that also acquired resistance is of importance in biofilm resistance, are at present speculations.

2.4 Effectiveness of microbicides and the duration of their effect

Mode of action and effectiveness of a microbicide are determined by the interplay of the chemical and physical properties of the active ingredient molecule, which in turn depend on the molecular structure. The properties involved include water- and lipid-solubility, polarity, ionogenicity, degree of dissociation, partition factor, reactivity and stability. The differences in modes of action should always be considered as they may result in different consequences for the application of microbicides.

The interaction of membrane-active agents with microbial cells is generally a reversible process which does not lead to any inactivation of the microbicide other than transitional adsorption. The active ingredients remain intact and retain their long-term effect when at an effective concentration ($C_{\text{eff.}}$). Electrophilically active microbicides react as described with nucleophilic components of the microbial cells and in consequence are soon exhausted, i.e. biodetoxicated. The duration of their effect ($T_{\text{eff.}}$) is therefore limited and by nature dependent on the germ count encountered by the active ingredient. It also depends on the composition, temperature and pH of the medium, i.e. the concentration of the active ingredient decreases with time in relation to the size of the angle α as shown in Figure 19. In the case of electrophilically active microbicides a concentration much higher than $C_{\text{eff.}}$ is required to achieve a sufficiently long-term effect.

**Figure 19** Activity duration ($T_{\text{eff.}}$) of microbicides in dependence of the slope α and the microbicide concentration C_1 or $C_2 > C_{\text{eff.}}$

2.5 Summary

Summarizing it can be stated that suitable microbicides must have

1. one or several toxophoric structural elements which enable an active ingredient to cause a damaging event at one or more specific target sites on or in the microbial cell,
2. physicochemical properties to penetrate to the specific target sites,
3. optimal physicochemical properties with regard to their applicability on/in materials or in processes to be protected from biodeterioration,
4. tolerable toxicity, also with regard to the environment.

The development/synthesis of microbicides (see chapter 3) consequently calls for intercommunication of different disciplines. Microbiology and biochemistry inform the synthesizing chemist by providing information about the architecture of the microbial cell, by definition of target sites at which microbicides interact and by screening of potential active ingredients. Engineering checks the applicability of effective substances. Toxicology carries out toxicological examinations and evaluation of the toxicity data with regard to the application. Finally, leading structures are optimized in cooperation of the disciplines involved.

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3 R&D in material protection: new biocides

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3.1 Introduction: situation of R&D in material protection

Active ingredients are without any doubt the most important factor of success in the material protection business. Basically, the whole business relies on the availability of biocides which combine high efficacy and good application properties on the one hand with low cost and promising ecotoxicological properties on the other hand.

However, looking into the marketplace you have to realize that a lot of the important market products of today are based on relatively “old” biocides which have been introduced into the market many years ago.

What are the reasons? First of all, the life cycle of active ingredients in material protection is certainly much longer compared to active ingredients used in pharmaceutical or agrochemical applications.

More important are the increasing R&D and registration cost which are necessary to find and develop new biocides which will meet the high standards required nowadays.

In the future this situation will even become more critical especially in Europe as a result of the Biocidal Product Directive (BPD), a EU-wide regulation requiring extensive testing of all biocidal products before they are registered for sale. Both old and new products will have to be registered, and the registration cost including all toxicological tests are estimated at 3–4 million € for each active ingredient. Under these circumstances, producers will have to decide for which actives such an investment will be justified.

In the context of R&D, the BPD will have two major consequences:

1. Decrease in number of biocidal actives available to customers. There are estimations that this number will decrease in Europe from 1500–2000 actives today to less than 300–500 actives in the future.
2. Decline in new product introductions, because initial cost will be much higher and the return on investment will take longer.

At the same time, there will be increasing pressure to substitute compounds which give rise to concerns regarding their toxicological or ecotoxicological properties. Examples could be arsenic-based products, formaldehyd-releasing compounds or tributyltin-(TBTO)-based antifoulants.

The situation of R&D in material protection thus is characterized by a double challenge: Increasing demand to substitute existing “old” products, but at the same time increasing R&D and registration cost for new biocides.

Under these circumstances, many competitors have in fact reduced their R&D efforts to find completely new biocides and have restricted their R&D to small chemical variations of their own “old” biocides and to the development of new formulations using combinations of existing biocides. Bayer is one of few companies still actively pursuing research for new biocides.

In this chapter, some results from R&D for new biocides obtained during the last 10–15 years will be presented. New fungicides, bactericides, insecticides and antifouling compounds will be discussed. In addition, new approaches like the use of enzymes in material protection, new technologies for finding new molecules and the testing of material protection products will be covered briefly.

3.2 Fungicides

The search for new antifungal molecules for material protection is often linked to the research for new antifungal compounds in plant-protection and medicine. In all applications the intention is to kill or inhibit fungi to prevent damage caused by them.

Table 1 Market Introduction of important Biocides.

Active ingredient	Application	Year of Introduction
p-Chloro-m-cresol (PCMC)	Bactericide	~ 1920
Formaldehyde – releasing compounds	Bactericide	~ mid 1930s
Quats (Benzalkoniumchloride)	Bactericide	~ mid 1930s
Isothiazolinones (e. g. Benzisothiazolinone)	Bactericide	~ end 1960s
Sulfenamides (e. g. Dichlofluanide)	Fungicide	~ end 1960s
Iodopropargyl-Butylcarbamate (IPBC)	Fungicide	~ 1970

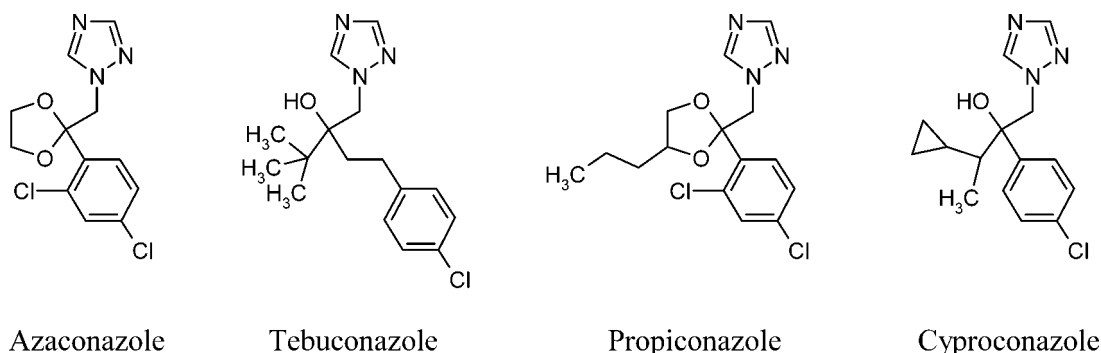


Figure 1 Triazole Fungicides used in Material Protection.

In agriculture the intention is to control phytopathogenic fungi to prevent losses in crop yield and quality, in medicine to prevent and cure diseases caused by pathogenic fungi. The aim of fungicides in material protection is to prevent economical losses by biodeterioration and degradation of wood, paints and other technical materials.

Although in all three indications fungi are involved, the requirements concerning the spectrum of activity, physicochemical properties and economical aspects are different.

The major goal in antimycotics research is to find systemic highly specific antifungal drugs for deep-seated mycoses without side effects for the patients. The focus in agricultural fungicide research is toward systemic fungicides which act as inhibitors of fungal specific targets. Additionally, a persistence in the environment is undesired.

In material protection however, a broad spectrum of antifungal activity is necessary. This broad activity can only be achieved with active ingredients acting as multiside-inhibitors (except wood preservatives against wood-destroying fungi). Compared to agrochemical fungicides, a good longterm-stability, a high stability toward alkaline hydrolysis and a good compatibility to many, very different technical systems is necessary.

3.2.1 Azoles [II, 14.]*

The class of azoles (Imidazoles, Triazoles) is one example for a large group of actives which have a fungal specific target. Intensive research over the last 30 years led to several well established compounds as antimycotics for human- and veterinary uses and fungicides for different agrochemical uses. In the 1970s and 1980s, the triazoles were by far the most rapidly growing class of fungicides. The azoles found have very different chemical structures.

Azoles are acting as C¹⁴-demethylation-inhibitors (DMIs) in the biosynthesis of ergosterol, a characteristic sterol in many higher fungi as Ascomycetes, Basidiomycetes and Fungi imperfecti. The qualitative changes of sterol composition (e.g. presence of C-14 methyl sterols) in plasma membranes containing high levels of sterols probably leads to an altered membrane fluidity. The result is a delay in fungal growth or in higher concentrations a fungicidal effect (Buchhauer, 1987; Scheinpflug, 1987).

The high activity of azoles against plant pathogenic basidiomycota, which are similar to wood-destroying fungi, resulted in an intensive research to use this class also for wood-protection.

Nearly all development candidates and commercially available triazoles and imidazoles were tested in in-vitro-tests against brown- and white rot fungi, wood-discolouring fungi and moulds. Only a small number of them, e.g. Azaconazole, Cyproconazole, Bromuconazole, Hexaconazole, Metconazole, Propiconazole, Tebuconazole and Tetraconazole, showed nearly the desired performance against the full spectrum of relevant basidiomycota for wood protection. So far only Azaconazole, Propiconazole, Tebuconazole and Cyproconazole were introduced into the market.

Azaconazole, the first azole introduced into the Market by Janssen, shows good activity against *Gleophyllum trabeum*, *Poria placenta* and *Coriolus versicolor*, but has a gap in the activity against *Coniothoria puteana*. So it needs higher concentrations in the final formulation or combinations with other ingredients. In combination with quaternary ammonium salts, it is also suitable for industrial wood preservation. Propiconazole, Tebuconazole and Cyproconazole have excellent activity in solvent as well as water based formulations against wood-destroying basidiomycota. All of these azoles are resistant to leaching (EN 84), evaporative aging (EN 73), are UV-stable and have a good stability in treated wood and formulations and therefore they are suitable for long lasting protection of wood against decay fungi (Buschhaus, 1992; Goodwine, 1990; Leclercq, 1983).

Combinations of Tebuconazole and Propiconazole provide a synergism that is interesting, for example, for industrial wood preservation (Buschhaus and Valcke, 1996).

*See Part II — Microbicide Data

3.2.2 Multiside inhibitors

The majority of fungicidal structures published for material-protection in the last years belong to the group of multiside-inhibitors. Most of them are electrophilically active agents.

The fungicidal and antibacterial activity is the result of a reaction with nucleophilic components in the microbial cell. The nucleophilic partners are thio-, amino- and amido-groups of amino acids in proteins. A reaction with the nucleophilic group leads to an unspecific enzyme inhibition which results in cell death. See chapter 2.

The biocidal spectrum of these compounds is generally broader compared to compounds with a specific mechanism. The unspecific action leads in general to a significant decrease in possible resistance (except resistance caused by reduced uptake and detoxification). The selectivity toward different organisms of these intrinsically unspecific compounds results from different transport-mechanisms and differences in the metabolism. The disadvantage is sometimes a higher toxicity toward warm-blooded animals and an increase in the possibility of other toxic effects like sensitization.

The reactive principle of most multiside-inhibitors found in the last years are activated C-C or C-N-double-bonds, reactive S-N-groups or aldehyde units.

The class of benzothiophene-2-carboxamid-S,S-dioxides came to the attention of the Bayer research because of their in vitro antifungal activity against a broad range of agro- and material-protection relevant fungi (Elbe et al., 1992; Elbe et al., 1996).

Structure-variation showed that the benzothiophene ring system, which is oxidized to the sulfoxide/sulfone, is absolutely necessary for the activity. Substituents at position 3 (R^2) of the benzothiophene diminish the biological activity, whereas aryl amides are very similar in activity compared to alkyl amides but have a slightly yellow colour. Alkyl esters show no enhanced activity compared to amides, but are less stable.

Taking together the biological activity, the physicochemical and toxicological data, N-Cyclohexyl-benzothiophen-2-carboxamid-S,S-dioxid was the best candidate for the use in waterbased emulsion paints and was developed as Preventol[®] TP OC 3082 and TP OC 3061 by Bayer. As can be seen from Table 2, it has a broad fungicidal and a slight algicidal activity.

The active ingredient is colourless, has a very low vapour pressure and an excellent toxicological profile.

Tests performed in different paints, including artificial weathering, showed that the active ingredient is suitable for dispersion paints in concentrations ranging from 0.4 to 0.7% (Table 3). Additional tests showed the suitability for some polymers and for antifouling paints (Kunisch et al., 1997).

Due to the low water solubility the leaching rate is low. Under the influence of light no formation of coloured products is initiated.

The active principle of cyaniminodithio compounds is an activated C-N doublebond.

A large number of these compounds were published, depending on the substituents, as herbicides, insecticides, fungicides and bactericides or for the use in material protection.

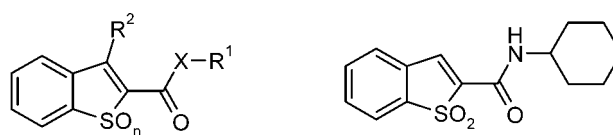
Cyaniminodithiocarbonates with chloromethyl substituents were published for the use as fungicides in plant protection and solvent based wood protectants (Walek et al., 1986; Walek et al., 1990a; Walek et al., 1990b). Methylen-bis(methyl-cyaniminodithiocarbonate) is suitable as a protectant for silicone based sealing compounds (Thust et al., 1986).

Because of their instability toward alkaline media and the occurrence of unpleasant odours, their applicability is limited to neutral or acidic media.

Based on these results, broad chemical variations were carried out to overcome these effects.

The breakthrough came with a new method to synthesize cyaniminodithio compounds with a direct linked S-Aryl group by the reaction of alkylcyanimidothio-salts with diazonium salts (Walek et al., 1993; Walek et al., 1996). Their in-vitro results against bacteria and fungi are in the range of the above mentioned compounds, but their stability toward alkaline media is better, so the range of the potential applicability is larger. The variability of the S-Aryl group is large, and S-Hetaryl groups lead also to active ingredients (Table 4).

Structure variations showed that an exchange of the C-N-Group against SO_2 in N-Sulfonyliminothio or dithiazoldioxide compounds is possible (Uhr et al., 1997; Uhr et al., 1998). Alkyl or aryl-substituents at the



Preventol TP OC 3061

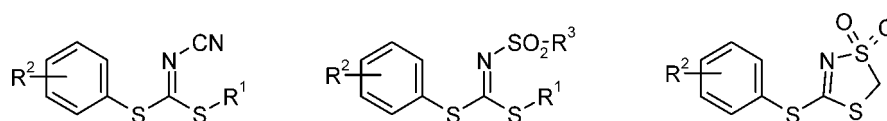
Figure 2 Benzothiophene-2-carboxamides.

Table 2 Biological spectrum of Cyclohexyl-benzothiophen-2-carboxamid-S,S-dioxid (Preventol[®] TP OC 3082) in ppm.

Fungi		Algae	
<i>Alternaria tenuis</i>	50	<i>Oscillatoria tenuis</i>	50
<i>Aspergillus niger</i>	75	<i>Chlorella vulgaris</i>	10–20
<i>Aureobasidium pullulans</i>	15		
<i>Chaetomium globosum</i>	75		
<i>Cladosporium herbarum</i>	75		
<i>Paecilomyces variotii</i>	50		
<i>Penicillium brevicaulis</i>	50		
<i>Sclerophoma pityophila</i>	5		

Table 3 Mould resistance of Preventol[®] TP OC 3082 in exterior paints.

Paint formulation	Guide value for dosage ¹
Polyvinylacetate (PVA) ²	0.4%
Styrene acrylate ³	0.4%
without ageing	0.7%
250 h weathering	
Pure acrylic ³	0.4%
without ageing	0.4%
250 h weathering	

¹ calc. on finished paint (Lab. result)² run by Bayer³ run by Paint Research Association UK according to BS 3900**Figure 3** Iminodithiocarbamate compounds.

N-atom lead to a loss of activity or to a switch to other biological mechanism as in Buthiobate (sterol biosynthesis inhibitor).

In styrene-acrylate based emulsion paints, 4-Methoxy-phenyl methyl cyanodithioimidocarbonate shows a good activity down to 0.3% active ingredient in the paint. N-Sulfonyliminodithio compounds and dithiazolidioxides are slightly less active in this test.

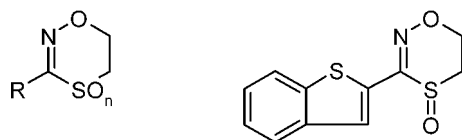
Due to the activated C-N-doublebond of the oxathiazines, they probably have a multiside inhibition.

3-Aryl-5,6-dihydro-1,4,2-oxathiazines and their oxides are known since the early 1980s (Brouwer et al., 1984). They are published as herbicides, fungicides, plant dessicants and defoliants in agricultural and biocidal applications, but no compound was developed for these indications (Figure 4).

In the mid 1990s this class came again into focus, when good activities against bacteria and material protection relevant fungi were found.

Table 4 Antifungal in-vitro-activity (MHK) of Cyaniminodithio-, N-Sulfonyliminodithio-, Dithiazolidioxide-compounds in ppm.

<i>Aspergillus niger</i>	10	100	100
<i>Chaetom. globosum</i>	5	20	100
<i>Penicillium brevicaulis</i>	10	20	100
<i>Sclerophoma pityophila</i>	5	10	5
<i>Trichoderma viride</i>	50	100	> 500
<i>Cladosporium herbarum</i>	20	50	5
<i>Alternaria tenuis</i>	5	20	< 1
<i>Aureobasidium pullulans</i>	5	20	5



Bethoxazine

Figure 4 5,6-Dihydro-1,4,2-oxathiazines.

A great variability of substituent R can be seen from Table 5 (Davis et al., 1998). An oxidation of the sulfur in the oxathiazine ring system to the sulfoxide/sulfone seems absolutely necessary for the biological activity.

In practical tests, the threshold concentrations against blue-stain and mold in a stick-test (50% ethanol solution) are ranging between 50 and 1000 ppm (*Aureobasidium pullulans*, *Aspergillus niger*, *Sclerophoma entoxylina*) and 1000 to 5000 ppm (*Trichoderma viride*).

Furthermore an activity against fresh-water and marine algae as well as aquatic organisms like *Artemia* in the range of 2.5 to 10 ppm was observed for Bethoxazine and makes it interesting for the use in antifouling paints (Van Gestel, 1998).

3-(Benzo[b]thien-2-yl)-5,6-dihydro-1,4,2-oxathiazine 4-oxide (Bethoxazin) was developed by Janssen for wood protection, antifungal and antifouling paints.

A synergistic effect of Bethoxazin with Preventol[®] TP OC 3061 was observed in the poison plate test with *Aureobasidium pullulans*, *Aspergillus niger*, *Sclerophoma entoxylina* and *Trichoderma viride* and in a stick test (Scots pine) with *Aureobasidium pullulans*, *Aspergillus niger*, *Sclerophoma entoxylina* and *Penicillium purpurogenum* (De Witte et al., 1999).

Table 5 Antifungal in-vitro-activity (MHK) of Oxazines in ppm: Variation of the aryl substituent R.

<i>A. niger</i>	5	5	< 1	5	2,5
<i>A. versicolor</i>	2,5	5	2,5	2,5	2,5
<i>A. pullulans</i>	25	25	10	25	10
<i>G. candidum</i>	25	25	10	10	25
<i>T. viride</i>	25	25	10	25	10

3.2.3 Chelating agents

Thiocarbamoylpyrazolones are new lead structures with a broad spectrum of antifungal activity (Heuer et al., 1996; Sasse et al., 1992). They are not acting as electrophiles, but are probably acting as chelating agents.

The variation of substituents showed a broad variability in R⁴, whereas R¹ and R² should be hydrogen and R³ is limited to hydrogen and methyl.

In styrene-acrylate based emulsion paints they are active down to 0.3%, but their main disadvantage is their leaching from the paint film. Trials to overcome the leaching by increasing the lipophilicity lead to Thiazolylpyrazolones (Heuer et al., 1995). The in-vitro activity toward fungi and the structure-activity relationship is similar to Thiocarbamoylpyrazolones.

In polyvinylacetate as well as styrene-acrylate based emulsion paints, they are active in the range of 0.3% to 0.6%. After a 24 h leaching, the activity decreased to 1%. Evaporative aging gave no decrease in activity.

The ability to form strong coloured complexes with heavy metal led to a stop of the development.

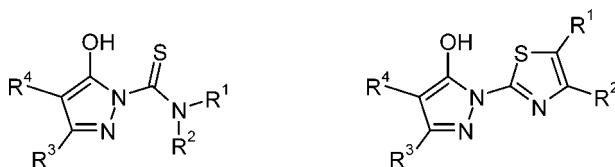
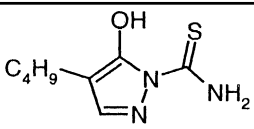
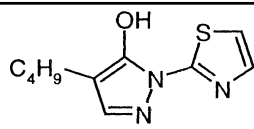


Figure 5 Thiocarbamoylpyrazolones.

Table 6 Antifungal in-vitro-activity (MHK) of 1-Thiocarbamoyl-5-hydroxy-pyrazoles and 1-Thiazolyl-5-hydroxy-pyrazoles in ppm.

		
<i>Aspergillus niger</i>	50	< 50
<i>Chaetom. globosum</i>	10	< 50
<i>Penicillium brevicaulis</i>	20	< 50
<i>Sclerophoma pityophila</i>	15	10
<i>Trichoderma viride</i>	20	50
<i>Cladosporium herbarum</i>	20	50
<i>Alternaria tenuis</i>	5	10
<i>Aureobasidium pullulans</i>	5	20

3.3 Bactericides

Bactericides for material protection are necessary for in-can/in-tank preservation of aqueous functional fluids, for example polymer emulsions, paints and coatings, adhesives and sealants, mineral slurries, metal working fluids, cosmetics and personal care products and cooling and recreational water, since every material which comprises of water and organic material as nutrient can be deteriorated by microorganisms. The decomposition leads not only to loss of quality and functionality causing problems in industrial processes, but also to human health hazards. Due to the great variety of materials with their varied chemical and physical properties, a large number of bactericides were developed and are marketed today. Since many different bacteria (gram negative and gram positive) are able to attack material, it is necessary that the deployed bactericide has a broad range of activity. Therefore bactericides used in material protection have an unspecific rather than a specific mode of action. In principle, all bactericides are divided into three classes according to their mode of action:

- membrane-active substances which interact with the membrane of bacteria, causing leakage of intracellular constituents, thus leading to cell death,
- electrophilic compounds, which react with nucleophilic cell entities such as amino acids, proteins and enzymes, thus irreversibly inhibiting vital cell functions, and
- chelating agents which exert their antimicrobial effects by chelatisation of metal ions which are essential for the function of cell metabolism (Paulus, 1993).

Today, there is pressure on some bactericides due to environmental concerns and human health hazard and only three points of discussion should be briefly mentioned here. First, the use of bactericides containing chlorine or bromine in the molecule is discussed in Europe because of AOX problems in waste water treatment. Second, there is an increasing pressure on bactericides based on formaldehyde or formaldehyde releasing compounds due to health hazards. The third issue is the R43 labeling (sensitizing through skin contact possible) of products which contain more than 15 ppm CMIT/MIT in a three to one ratio in Europe.

Out of these reasons, there is a real market need for new active ingredients for in-can preservation. A new bactericide has to fulfil the following requirements: high efficacy against a broad range of microorganisms, thus, low application concentration; compatibility with ingredients of the final product, for example amines; high heat and pH stability; effectiveness over a wide pH range; low toxicity to humans and other non-target organisms; no mutagenic and no sensitizing effects; low volatility; high water solubility and ease of application.

The research performed during the last 10–15 years in different companies in the area of bactericides for industrial preservation has been focused mainly on two aspects: To find and develop new chemical entities or to improve the manufacturing processes of already marketed bactericides.

3.3.1 Membrane-active compounds

Biocides acting by interfering with the membrane of microorganisms are very well known. Phenolics, alcohols, acids and quaternary ammonium salts belong to this class of compounds. These compounds are commodities and because of that not much research has been done in this area. Only para-Chloro-m-cresol [II, 7.3.1.)] (Figure 6), which is an important bactericide with a broad activity against bacteria but also against mould and yeast, should be mentioned here. PCMC is used for the preservation of metal working fluids, pigment and filler slurries, concrete additives, paper and coatings etc.. It acts through interacting with the membrane of the bacteria leading to disruption of the membrane and, thus, to death of microorganisms (Paulus, 1993).

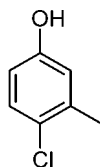


Figure 6 PCMC.

Since the usage of PCMC in Europe is getting more difficult due to AOX problems in waste water treatment, despite the fact that the biodegradability of PCMC has been proven (Bayer AG, 1998), our research groups examined whether the chlorine atom in PCMC is necessary or whether a substitution of chlorine is possible. We found that a chlorine substitution by numerous functional groups also led to active ingredients, which were however not as active as PCMC. Also no compound had the well-balanced activity spectrum of PCMC. Therefore it can be concluded that the chlorine function in PCMC is necessary for its activity (Bayer AG, 2001a).

3.3.2 Electrophilic substances

The electrophilically active substances have as toxophoric constituent an electrophilic group which is responsible for the antimicrobial effect, because it enables these active substances to react with specific nucleophilic entities of the microbial cell (Paulus, 1993). Examples of this class are aldehydes [II, 2.], e.g. Glutaraldehyde, compounds with activated halogen atoms [II, 17.], e.g. Bronopol, and microbiocides with an activated S-N-bond [II, 15.], for example Isothiazolinones. Since Isothiazolinones represent a major class of bactericides for industrial preservation, much research has been performed in this area.

A bio-isosterism between a carbonyl group and a sulfur or a sulfynyl group in active ingredients is often discussed. Therefore the examination of 1,3,2-Benzodithiazoles and 1,3,2-Benzodithiazole-S-oxides (Figure 7) as sulfur respective sulfur oxide analogues of BIT [II, 15.6.] (Figure 8) was a very interesting task. Abbott has reported certain 1,3,2-Benzodithiazole-S-oxides claiming their application against mycosis (Klein et al., 1991; Klein et al., 1994). We found that Benzodithiazoles and Benzodithiazole-S-oxides exhibit good antibacterial effects in vitro if R represents a carbon chain with up to four carbon atoms or a phenyl substituent (Uhr et al., 1995). Due to the low water solubility of the non oxidized substances, it was however impossible to transfer the in-vitro activity into a sufficient effect in application tests. However, the sulfur-oxides showed a broad activity in application tests, and the derivative with R = Methyl reached the antibacterial effect of BIT and exhibited even better activity against mould and yeast than BIT. In contrast to reported results (Klein et al., 1994), we found also good antibacterial activity if R represents long alkyl chains which were substituted by polar substituents, especially disubstituted amines.

The influence of a N-Alkyl chain on the activity of the bactericide BIT has been studied in detail by different companies (Figure 9). It is obvious from the published results, that with increasing chain length a shift of the activity profile occurred, namely from the antibacterial agent BIT to compounds with stronger fungicidal effects.

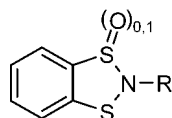


Figure 7 1,3,2-Benzodithiazoles (n = 0) and 1,3,2-Benzodithiazole-S-oxides (n = 1).

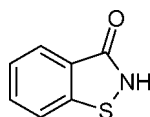


Figure 8 BIT.

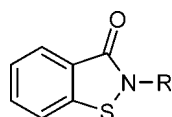


Figure 9 N-Alkyl-Derivatives of BIT.

N-alkyl derivatives with up to three carbon atoms have better antifungal activity than BIT (Buckley et al., 1978), and N-alkyl compounds with an alkyl chain between six and eight carbon atoms showed better in-vitro results than the corresponding short chain derivatives (Lindner et al., 1992). The authors emphasized that the long chain derivatives were fewer soluble in water, so that these compounds showed a better leaching performance and thus allowed long term protection of coating films. Contrary to the just mentioned results, WO 96/22023 described structure activity relationships regarding plastic destroying fungi and showed that compounds with a short alkyl chain exhibit slightly better MIC values than the long chain derivatives (Austin, 1996). After watering however, the longer chain derivatives were again more effective. Today n-Butyl-BIT [II, 15.7.] (BBIT) (Figure 10) is marketed as fungicide for the preservation of plastics, paints etc..

Recently, Zeneca introduced a new active ingredient based on isothiazolinone chemistry, namely 4,5-Trimethylene-2-methyl-isothiazolinone [II, 15.8.] (Figure 11). The published MIC values against mould, yeast and bacteria are within the range of other bactericides of this chemical class (Pommer et al., 1993). Zeneca showed that in application testing their new active ingredient was more effective than BIT in the preservation of water based paints based on polyvinylacetate or acrylate (Pommer et al., 1993). Isothiazolinones are often sensitive to degradation through amines thus resulting in a slow decrease of the protection efficiency over time. However, it was published that 4,5-Trimethylene-2-methyl-isothiazolinone is more stable against amine degradation than other active ingredients of this class (Eacott, 1991). The manufacturing process was well examined and several optimizations have been claimed (Shrott et al., 1982; Maignan et al., 1986; Moffatt, 1991a; Moffatt et al., 1991b). Zeneca has performed much research on structures related to their new active ingredient, and a few bactericidal structure activity relationships have been published. It was shown that open chain disulfides (Figure 12) with amide or N-methylamide group have moderate biocidal in-vitro activity whereas the activity strongly decreased with increasing length of the N-alkyl chain (Austin et al., 1995). Furthermore the utilization of several thiosulfonic acid derivatives and their salts (Figure 13) as antimicrobial agents was claimed (Austin, 1992). But again, the activity strongly depends on the length of the alkyl chain and only the N-methyl derivative has noteworthy biocidal effects. Probably due to high production cost, the active ingredient 4,5-Trimethylene-2-methyl-isothiazolinone has been not marketed up to now.

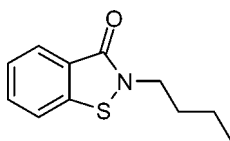


Figure 10 n-Butyl-BIT.

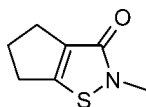


Figure 11 4,5-Trimethylene-2-methyl-isothiazolinone.

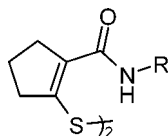


Figure 12 Disulfide derivatives.

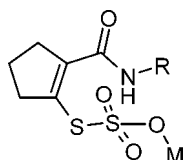


Figure 13 Thiosulfonic acid derivatives (M = metal).

Triclosan [II, 7.6.1.] (Figure 14) is a broad spectrum antibacterial agent which is especially used to prevent spoilage of consumer care products like soaps, deodorants and toothpastes. It is also used in other application fields, for example in the antimicrobial fittings of fibres and plastics. As a result of the broad activity of TCS, it was generally accepted that TCS has an unspecific mode of action (Paulus, 1993). However, recently academic research groups proved that TCS interacts with an essential enzyme of the fatty acid biosynthesis. Thus, a specific rather than an unspecific mechanism of action is discussed (Heath et al., 2000). This finding has led to increasing public concern regarding antibacterial resistance development (Levy, 2001). The possible formation of dibenzofurane derivatives during the production process as well as during the combustion of waste which was originally protected by TCS, together with the enrichment of TCS in the environment due to high lipophilie in combination with poor biodegradation, is another matter of public discussion (Adolfsson-Erici et al., 2000; Bundesumweltamt, 2001). Recently, Ciba has patented a new manufacturing process in which the formation of dibenzofurane derivatives as side products is excluded. They found that the hydroxyl group can be introduced by Baeyer-Villiger-Oxidation of a methyl ketone and subsequent hydrolysis instead of decomposition of a diazonium salt, as it is outlined in Figure 15 (Kulkarni et al., 1998). Another aspect of Ciba's research activities is the development of new active ingredients as potential back-up substances for TCS. First Diclosan [II, 7. 6.2.] (Figure 16) was introduced which has MIC values in the range of TCS (Ochs et al., 1999; Ochs et al., 2000). Recently Ciba published efforts to find halogen-free TCS analogues. They focused on alkyl substituted phenoxyphenol derivatives, but however, none of the described substances reached the broad and high activity of the standard TCS. The best MIC values were obtained for 4-(2-tert.-Butyl-5-methylphenoxy)phenol (Hölzl et al., 2000).

3.3.3 Chelating agents

Long-term chelating of metal ions, which are essential for the proper function of several enzymes, represents another main principle of action of biocides with the result that vital metal ions are not longer available for the organism and essential processes are inhibited. A research group of ICI has been working in this area and claimed thiohydroxamic acids and metal complexes thereof as biocides for industrial preservation (Figure 17) (Austin, 1987; Austin, 1990). The reported data showed that the open chain derivatives have always poorer

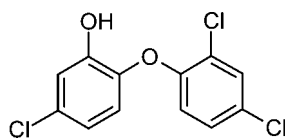


Figure 14 Triclosan.

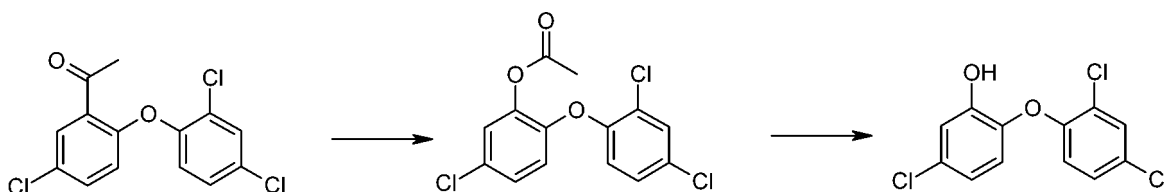


Figure 15 New manufacturing process for Triclosan.

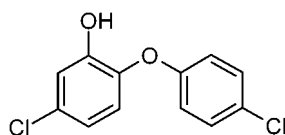


Figure 16 Diclosan.

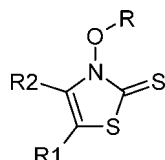


Figure 17 Thiohydroxamic acids.

MIC values than the cyclic substances, probably because of lack of chemical stability. The analysis of the MIC values of the cyclic derivatives shows that compounds with R unequal hydrogen have MIC values well below the corresponding parent compounds with R representing hydrogen. The reason for this effect is probably the poorer ability to generate stable metal complexes, since the donor capability of the oxygen is decreased. It is furthermore noticeable that additional substituents at C5 or substituents at C4, which are bulkier than methyl, lead to decreasing activity especially against mould. The substance with the best MIC performance is the zinc complex with R1 and R2 representing H and Methyl, respectively. The use of these complexes for in-can preservation may be problematic due to the poor water solubility of the compounds. Another aspect of concern is the point that many metal complexes of this kind are coloured, leading to a discoloration of the protected medium. Probably due to these reasons, none of these compounds is marketed today.

3.3.4 *Inorganic bactericides*

The antibacterial effect of metal ions and especially silver, copper and zinc ions is well known. Silver and silver ions are used in medicinal treatments ranging from severe burns to Legionnaires Diseases. Silver-based products are also applied in water purification processes. Metal ions achieve their antibacterial effect by two mechanisms: First the metal ions influence the electrochemical potential between the internal and external parts of the cell, and second, after penetration of ions into the cell, they compete with other essential ions like magnesium, calcium and potassium and they aggregate with thiol groups of enzymes and proteins.

In material protection, silver based biocides are used mainly for the preservation of consumer products like household articles, textile products and pvc floors. The preservation of liquid products with silver-based ingredients is possible with systems which allow controlled release of silver ions. A controlled release systems is necessary, because, if the silver concentration in the liquid phase is too low, microorganisms will grow, but, if the silver concentration is too high, the product will get black. Therefore different releasing systems based on zeolite or titanium dioxide carriers or on polymers have been developed.

3.4 **Insecticides**

In the field of material protection, insecticides are used basically for the preservation of all kind of wood, for example buildings, cross ties, part of bridges, telegraph poles, pallets and containers to prevent the attack of different wood destroying insects. Some species of insects lay their eggs on or below the surface of wood and their larvae then feed and pupate in the wood, whereas other species do not eat wood, but destroy it by hollowing it out for nests. Another species worth mentioning are termites. Termites are locally present in Europe but in the United States, Japan, South Asia, Latin America and Australia they belong to the most economically important insects causing significant damage every year. There are several species of termites known and the most common ones are the subterranean, drywood and dampwood termites with the first mentioned being the most destructive one. Because subterranean termites are soil inhabiting insects living in complex colonies, the conventional control method is to establish an insecticide barrier between the termite colony and the wood structure in the soil. Another approach is to use strategically placed bait stations with attractive food and later with a termiticide. In the last years new insecticides for both approaches have been marketed.

Another field of application of insecticides in material protection is the preservation of plastics, for example the protection of cable covering from attacks by insects.

For many years the prevention and treatment of insects in wood protection relied heavily on the use of organochlorine and organophosphate insecticides which provided long-term protection. Due to toxicological and environmental concerns, these chemicals were largely withdrawn from use.

All insecticides used in material protection were originally developed for crop protection and later on tested, registered and marketed in this additional application. Suitable active ingredients for the use in wood protection have to fulfil the following requirements: high efficacy, thus, low application concentration; low toxicity to mammals and other non-target species; low water solubility and, thus, high resistance to leaching; low volatility and, thus, high evaporation resistance; long lasting efficacy, resistance to light and UV; heat stability; lack of color and wood-staining properties and ease of application. By far not all the insecticides marketed in crop protection fulfil these challenging requirements, so that each active ingredient has to be extensively tested for suitability.

3.4.1 *Pyrethroids*

In the beginning of the eighties, synthetic pyrethroid insecticides were introduced into the wood protection market substituting organochlorine and organophosphate insecticides. The pyrethroids share similar modes of

action. They are considered axonic poisons and they apparently work by keeping open sodium channels in neuronal membranes leading to death of insects. Pyrethroids are fast acting and act mainly as contact poison but also as stomach poison. Contrary to the organophosphate insecticides which act also as neuro-toxins, the toxicity of pyrethroids against warm-blooded organism is significantly reduced. Furthermore, pyrethroids do not accumulate in the environment. Therefore substitution of organochlorine and organophosphate insecticides by pyrethroids in material protection was a remarkable improvement concerning toxicological and ecotoxicological questions.

Some pyrethroids are especially useful for wood preservation due to their physico-chemical parameters, e.g. very low vapor pressure, high UV-resistance and very low water solubility, resulting in a long-term protection of technical material. Today the active ingredients Permethrin, Cypermethrin, Cyfluthrin, Deltamethrin and Bifenthrin (Figure 18 and 19) are used in wood protection. Due to their long-term protection and their fast action these compounds can be used in preservative and curative applications. Table 7 summarizes the efficacy in tests according to European standards EN 21 and EN 47.

3.4.2 Insect growth regulators

In the 1990s the first active ingredients of a new class of insecticides were introduced in wood protection: benzoylureas. This was really a remarkable innovation, because all insecticides used until then were acting as neurotoxins, and benzoylureas have no neuro-toxic effects at all. They are insect growth regulators (IGRs) and they deduce with chitin synthesis rather than being typical poisons that attack the insect nervous system. Their mechanism of action is by interaction with chitin, the major component of the rigid exoskeleton covering insects at any stage of the discontinuous development of insects, when they shed their integument and create a new one larger in size. Exposure of nymphs to benzoylureas causes improper attachment of the new cuticle during moulting and produces a cuticle, that lacks some of the layers that would normally occur. Treated larvae either are incapable of emerging from eggs or will die at the next moult because of rupture of the new malformed cuticle or from starvation. Treated adults lay non viable eggs. The active ingredients are slow acting stomach poisons without any noteworthy contact effects. The chemical and physical properties, especially the low water solubility, low vapor pressure, high heat stability and good stability against degradation by light and UV, allow the usage of benzoylureas in wood preservation (Wegen et al., 1996; Pallaske, 1998). Important examples are Diflubenzuron, Flufenoxuron, Hexaflumuron and Triflumuron (Figures 20 and 21). Table 8 summarizes published efficacy data of these compounds.

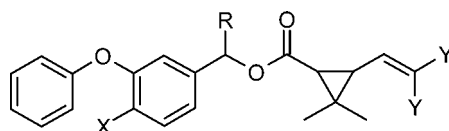


Figure 18 Permethrin (R = X = H, Y = Cl); Cypermethrin (R = CN, X = H, Y = Cl); Cyfluthrin (R = CN, X = F, Y = Cl); Deltamethrin (R = CN, X = H, Y = Br).

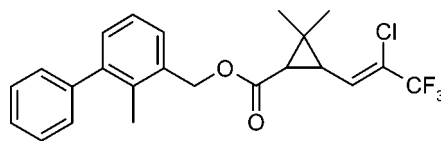


Figure 19 Bifenthrin.

Table 7 Efficacy data of Pyrethroids.

Compound	EN 21 [g/m ³]	EN 47 [g/m ³]	Reference
Permethrin	30–50	1.0–3.0	Grüning et al., 1986
Cypermethrin	20–30	0.3–0.9	Grüning et al., 1986
Cyfluthrin	8–12	0.3–0.5	Bayer AG, 2001a
Deltamethrin	4.6–9.1	0.4–0.6	Adam et al., 1996
Bifenthrin	7.1–9.8	0.052–0.1	Shires, 1996

EN 21: Method for the determination of the toxic values of a wood preservative against larvae of *Anobium punctatum* (de Geer), introduced into wood which has been treated previously by full impregnation.

EN 47: Method for the determination of the toxic values of a wood preservative against larvae of *Hylotrupes bajulus* (Linnaeus), introduced into wood which has been treated previously by full impregnation.

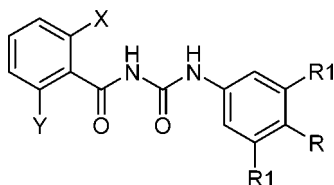


Figure 20 Triflumuron (X = Cl, Y = R1 = H, R = OCF₃); Diflubenzuron (X = Y = F, R1 = H, R = Cl); Hexaflumuron (X = Y = F, R1 = Cl, R = OCF₂CF₂H).

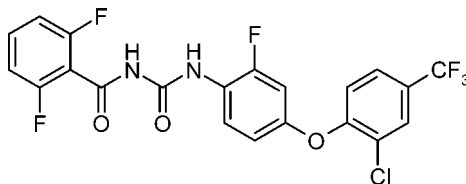


Figure 21 Flufenoxuron.

Table 8 Efficacy data of benzoylureas.

Compound	EN 21 [g/m ³]	EN 47 [g/m ³]	Reference
Diflubenzuron	> 1800	120–320	Wegen et al., 1996
Flufenoxuron	2–3 (48 weeks)	0.5–0.9	Wegen et al., 1996
Hexaflumuron	170	90–140	Wegen et al., 1996
Triflumuron	> 730	0.8–1.2	Wegen et al., 1996

EN 21: Method for the determination of the toxic values of a wood preservative against larvae of *Anobium punctatum* (de Geer), introduced into wood which has been treated previously by full impregnation.

EN 47: Method for the determination of the toxic values of a wood preservative against larvae of *Hylotrupes bajului* (Linnaeus), introduced into wood which has been treated previously by full impregnation.

Due to their slow acting effects and their lack of efficacy by contact, benzoylureas are useful for bait stations. Flufenoxuron and Diflubenzuron are active ingredients used in different recently introduced termite bait systems, whereas Triflumuron is applied directly to termite nests. Another completely different termite bait systems was developed based on a new stomach poison with delayed action called sulfluramid (Figure 22).

Another class of insecticides which interfere with the hormonal system of insects also belong to the group of insect growth regulators. These compounds have either juvenile hormone activity or act as ecdysone antagonists or agonists. By interfering with the hormonal system of insects, these active ingredients therefore produce deleterious effects on reproduction resulting in nonviable insects.

Fenoxycarb is a juvenile hormone analogue introduced to material protection (Figure 23). It acts by binding to juvenile hormone receptors and imitating their action by interfering with the moulting of early instar larvae, by inhibiting the metamorphosis to the adult stage and by affecting reproduction. It was published, that Fenoxycarb is very useful for the protective preservation of wood against wood borers (Valcke, 1995). Furthermore it has been reported, that Fenoxycarb has no efficacy against termites. However, today Fenoxycarb is the active ingredient in products which are used as fire ant bait.

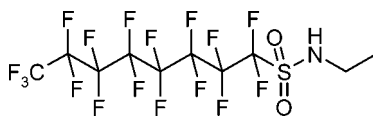


Figure 22 Sulfluramid.

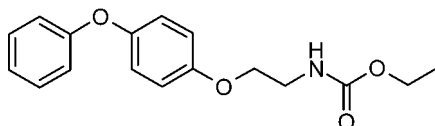


Figure 23 Fenoxycarb.

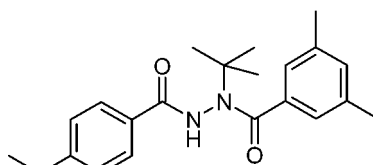


Figure 24 Tebufenocide.

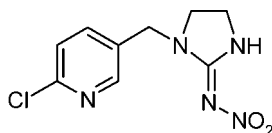


Figure 25 Imidacloprid.

Active ingredients belonging to the ecdysone antagonist class cause fail-regulation of developing stages, which are controlled by the skin-shedding hormone ecdysone. Treated larvae have to moult several times leading to nonviable organism. An example for this class is Tebufenocide (Figure 24). Up to now, only very few data concerning the efficacy of Tebufenocide in wood protection have been published (Pallaske, 1998).

3.4.3 Chloronicotinyle

A new class of active ingredients with a new mode of action against insects developed recently are chloronicotinyls. The first member of this class of insecticides which gained registration is Imidacloprid (Figure 25). The active ingredients of this class act on the central nervous system of insects by mimicking acetylcholine, thus causing irreversible blockade of postsynaptic nicotinic acetylcholine receptors leading to death of insects. Imidacloprid acts as contact and stomach poison. Due to imidacloprid's low vapor pressure, high stability against hydrolysis in acid as well as in basic medium and low water solubility, it is superbly suited for long term preservation of all kind of wood and wood products against attack by many different insects. It is especially active against termites (Tsuboi et al., 1992). The efficacy data of Imidacloprid against termites in comparison to Chlorpyrifos, an organophosphate insecticide, are shown in Table 9. Furthermore, Imidacloprid is useful as active ingredient in bait systems against cockroaches.

Crop protection companies have also developed further compounds belonging to the chloronicotinyl class. However, up to now none of these compounds are used for preservation of wood products against insect infestation.

3.4.4 Phenylpyrazole

Fipronil (Figure 26) is a new insecticide developed in the 1990s. It belongs to a new class of active ingredients called phenylpyrazoles. Fipronil acts by blocking the gamma-aminobutyric acid (GABA) regulated chloride channel of neurons in the central nervous system, resulting in neural excitation and death of insects. This active

Table 9 Efficacy of Imidacloprid against termites, Field Stake Test data, Gainesville, FL.

Retention (kg/m ³)		2-Year Termite Ranking *
Imidacloprid	Chlorpyrifos	
0.005	-	10
0.01	-	10
0.02	-	10
0.04	-	10
0.08	-	10
-	0.02	8.2
-	0.08	8.7
-	0.28	9.9
Control	Control	5.1

USA (Reference: Bayer, 2001b)

*: Termite Grading System according to AWP Standard E7-93

10 : Sound, 1 to 2 small nibbles permitted

9 : Slight evidence of feeding to 3% of cross section

8 : Attack form 3 to 10% of cross section

7 : Attack form 10 to 30% of cross section

6 : Attack form 30 to 50% of cross section

4 : Attack form 50 to 75% of cross section

0 : Failure

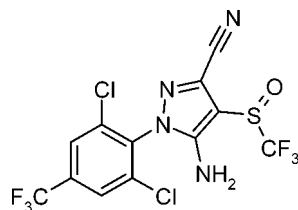


Figure 26 Fipronil.

ingredient is lethal to insects by contact as well by ingestion. Fipronil is used in material protection in soil treatment against termites and as bait against ants and roaches (Kimura, 1998).

3.5 Antifouling

Marine biofouling is the result of the growth of organisms on natural and artificial structures immersed in sea water. It causes serious problems in the shipping industry, in aquaculture and in cooling systems of power stations. Therefore, antifouling products are of significant economic importance.

When fouling organisms attach to ship hulls, oil platforms and pipelines, one faces severe difficulties. Ships suffer increased drag, leading to lower speeds and higher fuel consumption, increased cleaning costs and increased time out of service. Corrosion of pipelines escalates as many foulers produce corrosive metabolites. Also aquacultural systems have to deal with several problems due to fouling as fish and oyster cages become swamped by barnacles, mussels and algae.

The progress of biofouling on an unprotected surface immersed in sea water is quite well-known (Callow, 1990). After accumulating dissolved organic matter like proteins and polysaccharides in a so-called conditioning process, the surface is prepared for bacteria and diatoms to settle on it. Their subsequent multiplication and production of polysaccharides and adhesives forms a thin layer of organic matter – a biofilm – that works to trap more organisms, which are likely to be algal spores, marine fungi and protozoa. The final stage of fouling involves the settlement and growth of larger, visible marine invertebrates together with the growth of macroalgae. This last process is also called macrofouling and has species involved such as barnacles, serpulids, mussels and seaweed.

During the 1960s and 1970s the industry developed highly efficient antifouling paints using organotin compounds, namely tributyltin (TBT) [II, 19.5.]. With the development of self-polishing paints, having TBT chemically bonded to the copolymer, the leaching rate of these paints is controlled, because the tin compound is released when sea water hydrolyses the surface layer of the paint. In this way, it is possible for ships to stay in service for 5 years without repainting. This explains why TBT was the most widely used antifoulant.

Besides their toxicity to the target organisms, organotins can produce harmful effects on numerous marine organisms such as acute toxicity, bioaccumulation, decreased viability, and increased shell thickness (Maguire, 1987; Huggett et al., 1993). Being aware of these environmental impacts, the International Marine Organisation (IMO) has adopted a resolution which calls for the prohibition of the application of organotins in antifouling paints by January 2003 and for the complete prohibition of organotins in antifouling paints by January 2008. In addition other restrictions in several countries already apply. Therefore, the development of environmentally acceptable antifoulants is crucial for manufacturers of antifouling products. The ideal replacement will have a broad spectrum of activity against a diverse range of fouling organisms and provide a longterm protection without impacting non-target species.

3.5.1 Copper and organic biocides

Known for some hundred years as antifoulants, copper and copper compounds have reemerged in the wake of the restrictions applied to TBT. Copper is widely distributed in animal and plant tissue as an essential trace element and is by far not as toxic as tin. It is effective against a wide range of fouling organisms, but is particularly active against those species mainly responsible for hardfouling, namely sessile marine species like barnacles, mussels and serpulids. Nowadays copper containing coatings gain their efficiency from compounds like cuprous oxide, copper thiocyanate and copper bound to copolymers in an ablative resin system which slowly dissolves. The latter bringing along the benefit of taking with it all sort of early fouling stages while being hydrolyzed by sea water. Modern antifouling coatings might contain 10–50% by weight of copper ingredients depending on the vessels main region of service.

There are good reasons for the use of organic biocides as additional active ingredients in antifouling paints. Used alone, copper compounds lack efficient activity against early stage foulers like algae and diatoms. There exist a limited number of biocides, often referred to as secondary or booster biocides, which are particularly active against copper resistant organisms and therefore enhance the activity of copper compounds. In addition, as some of the biocides show activity against hardfoulers as well, the use of biocides can remarkably lower the

Table 10 Booster Biocides used in Antifouling Coatings.

Chemical structure	Chemical name	CAS-No.	Common name
	(1,3-Benzothiazol-2-ylsulfanyl)-methyl thiocyanate	[21564-17-0]	Benthiazole [II, 15.11.]
	4,5-Dichloro-2- <i>n</i> -octyl-4-isothiazolin-3-one	[64359-81-5]	Sea-Nine [II, 15.5.]
	<i>N</i> -[[dichloro(fluoro)methyl]sulfanyl]- <i>N'</i> , <i>N'</i> -dimethyl- <i>N</i> -phenylsulfamide	[1085-98-9]	Dichlofluanid [II, 16.5.]
	<i>N</i> -[[dichloro(fluoro)methyl]sulfanyl]- <i>N'</i> , <i>N'</i> -dimethyl- <i>N</i> -tolylsulfamide	[731-27-1]	Tolylfluanid [II, 16.6.]
	<i>N'</i> -(3,4-dichlorophenyl)- <i>N,N</i> -dimethylurea	[330-54-1]	Diuron [II, 10.9.]
	<i>N</i> -(<i>t</i> -butyl)- <i>N</i> -cyclopropyl-6-(methyl-sulfanyl)-1,3,5-triazine-2,4-diamine	[28159-98-0]	Cybutryne [II, 20.4.]
	2,4,5,6-Tetrachloroisophthalonitrile	[1897-45-6]	Chlorothalonil [II, 17.19.]
	2,3,5,6-Tetrachloro-4-(methylsulfonyl)pyridine	[13108-52-6]	TCMS pyridine [III, 17.12.]
	Zinc [bis(dimethyldithiocarbamate)]	[137-30-4]	Ziram [II, 11.11.3.]
	Zinc [ethylenebis(dithiocarbamate)] (polymeric)	[142-14-3]	Zineb [II, 11.12.2.]
	Zinc complex of 2-pyridinethiol 1-oxide	[3138-01-0]	Zinc pyriothione [II, 13.1.3b.]

copper content of such paints. This is of importance as with a growing concern that copper-based antifouling agents too could cause environmental problems, authorities have begun to review these effects. As a consequence, copper might suffer restrictions in future as well. An important issue and a limiting factor for future use of organic biocides will be the requirement for a rapid degradability in sea water. Especially the active ingredients Dichlofluanid, Sea-Nine and Tolylfluanid are rapidly hydrolyzed in sea water.

Table 10 shows the booster biocides used in antifouling coatings.

3.5.2 Natural toxins

Many marine species protect themselves from fouling by their own specific toxins. Several groups have reported molecules with highly efficient repelling properties, but in general their chemical structures are too complex for a commercial product and their toxicological profile is not suited to target a broad range of foulers (Keifer et. al, 1986; Fusetani et al., 1997; Jaspers, 1999).

3.5.3 Other concepts

Apart from using molecules which are biologically active against fouling organisms, there exist several approaches to keep sessile species from settling on surfaces by other means. Examples for some techniques are listed below:

3.5.3.1 Self-polishing coatings. Self-polishing surfaces continually lose their outer layers. This concept can be found in nature as it is used by some corals. They routinely slough their outer layers along with associated fouling (Vrolijk et al., 1990). The most effective tin-free marine antifouling coatings combine self-polishing properties with the release of toxic metals or organic biocides. Self-polishing properties are based on copolymers with hydrolyzable groups, mostly esters. (Figure 27). The ester hydrolyzes in sea water releasing his residue and leaving a polymer with a free carboxylic acid. Becoming highly water soluble by many carboxylic acid groups, the outer layer is eroded by abrasion taking with it all kinds of fouling. Thereby, a fresh layer of polymer surface is exposed allowing the process to be repeated.

Ablative coatings are most effective at high water velocities. Their lifetime is mainly determined by the temperature of the sea water and the thickness of the applied coating.

3.5.3.2 Fouling-release coatings. The basic idea behind the concept of the so-called foul-release coatings is to limit the strength of the joint between fouling organisms and surface. The bond has to be weakened in such a way that it can be broken either by the weight of the fouling itself or latest by the waters friction while the ship is moving. So far, mainly two technologies have evolved: Coatings based on fluoropolymers and coatings based on silicones (Brady, 2000).

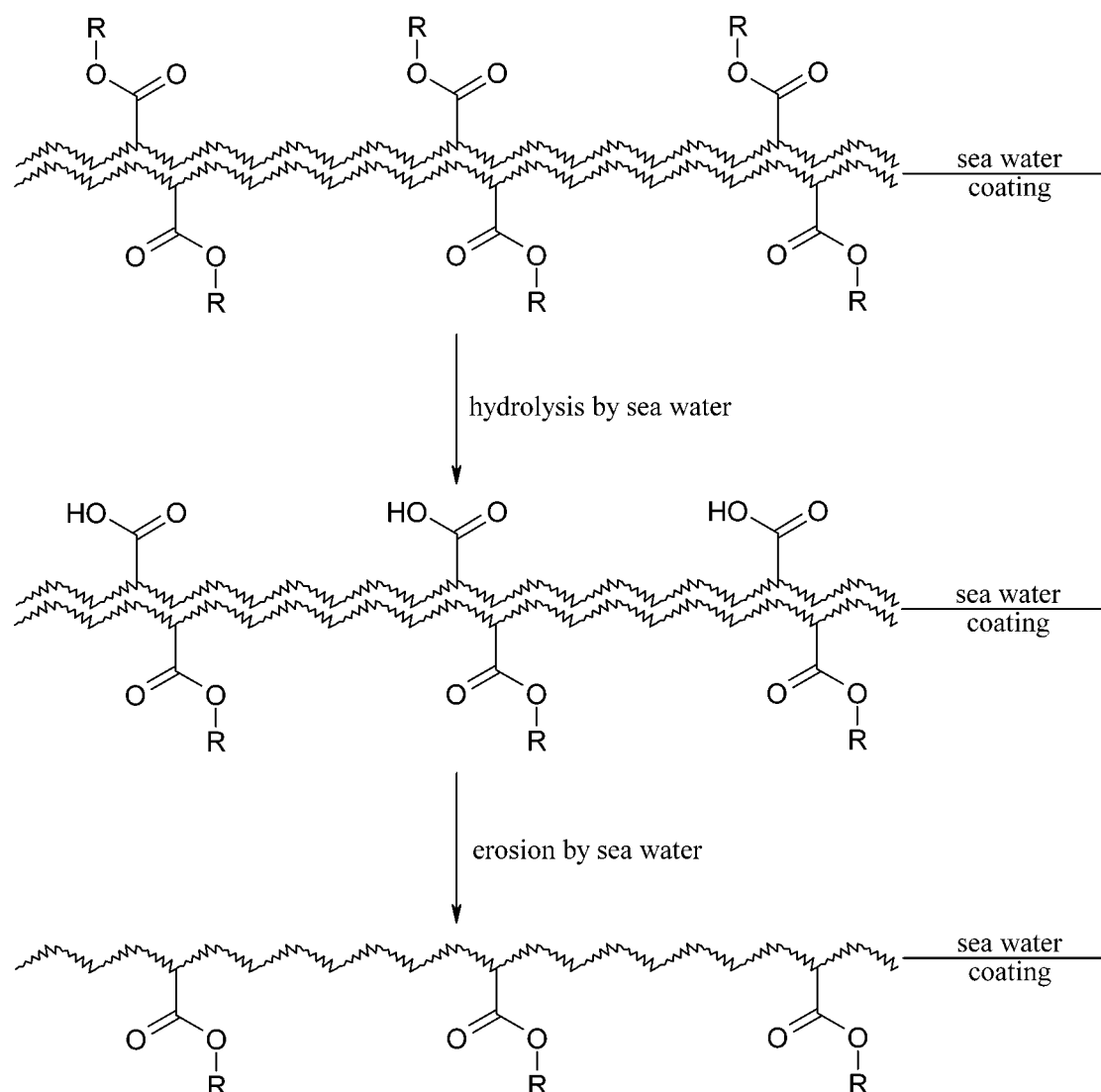


Figure 27 Principle of self-polishing coatings.

The chemical backbone of silicones used as non-stick coatings is mostly polydimethylsiloxane. The silicones have an unattractively low surface energy for most fouling organisms. An additional antifouling effect can be obtained through extruding oils, which are incorporated in the polymer, like silicone oil or paraffin. Silicones deform readily and release the fouling by a peeling mechanism that requires only low energy.

Fluorinated coatings achieve their effectiveness by discouraging the attachment of fouling organisms through an oriented surface of packed perfluoroalkyl groups having a very low surface energy.

3.5.3.3 Hydroviscous coatings. Another idea of protection follows the mechanism some fishes and algae use to avoid fouling (Rosen et al., 1971). They often cover themselves with a mucous layer which prevents organisms from reaching their living surface. On ships, the mucous layer is mimicked by a hydroviscous surface from absorbing water into its boundary layer, forming a type of fixed water-in-oil emulsion layer running along the hull. Since mucus as well as the artificial emulsion is almost water and hydrophilic, its resemblance to water might camouflage the surface. In addition to their potential antifouling properties, hydroviscous surfaces show potential for drag reduction.

3.5.3.4 Microtextured surfaces. It has been reported that cyprids show a tendency to leave surfaces with sharp ridges and grooves (Crisp et al., 1954). Patents from various companies for the approach of producing surfaces with fine hairs, grooves and ridges have been filed as well. These surfaces can be considered as mimics for the surface of plants or, in the case of a structure covered with fine hairs, the pelt of otters and seals. Microtextured surfaces and fiber-based concepts, however, need to be engineered in order to reduce their effect on drag.

3.6 New approaches

3.6.1 Antimicrobial enzymes

Enzyme use for biofilm control in water circuits has long been in the focus of discussions. This strategy is targeted to partly or completely replace organic biocides which are currently used. In particular the paper making industry is suffering from the formation of biofilms causing a variety of problems throughout the entire paper making process.

There are several enzyme formulations commercially available, but it seems crucial to obtain a good knowledge of which sort of biofilms are formed and which kind of polysaccharides and proteins are involved to choose the right enzymes. This is due to substrate specificities, pH and temperature dependence of enzymes.

As different species produce different polysaccharides within their biofilm matrices, it is obvious that one needs enzymes which are able to break a variety of chemical bonds. This is most likely with a blend of different enzymes which may require different conditions for optimum performance (Schenker et al., 1997). Today, a combined use of microbicides and enzymes appears to be most promising to develop an environmentally compatible and effective system.

But enzymes also become introduced to other applications in material protection. Oxidizing agents such as hydrogen peroxide or peroxy acids have a microbicidal effect due to their unspecific oxidizing power. Hydrogen peroxide as well as peroxy acids generate hydroxyl radicals which are highly reactive and responsible for the antimicrobial action. The pH for optimum efficiency is in the acidic range, as in alkaline media peroxy compounds decompose too quickly.

Enzymatic oxidizing systems are capable of generating reactive oxygen species as well. The system Myavert[®] C uses a balance of lactoperoxidase and glucose oxidase to yield preservation in cosmetic and toiletry formulations (Guthrie, 1992). For optimum performance a target pH of between 4 and 6 should be considered.

Other systems using peroxidases, haloperoxidases and laccases for use as preservatives of water based paints, cosmetics, contact lenses and personal care products have been claimed (Johansen et al., 2000; Miller, 2000; Johansen, 2001). As many of the mentioned enzyme systems have an optimum pH for their activity, it remains questionable whether these systems are useful for a broad range of application in material protection, in particular for those applications requiring alkaline conditions.

3.6.2 Biofilms (see also chapter 5.1)

Bacteria and other microorganisms in nature often exist as sessile communities called biofilms. These communities develop structures that are morphologically and physiologically differentiated from free-living microorganisms. They all have in common that the organisms are embedded in a matrix of extracellular polymers. In this matrix, they are better protected against biocides than individuals. Biofilms are ubiquitous in the environment and can cause problems such as biofouling or microbial corrosion in industrial processes (Flemming et al., 2001).

As environmental conditions often change rapidly, bacteria need to respond quickly in order to survive. These responses include adaptation to availability of nutrients, defence against other microorganisms which may compete for the same nutrients and, of particular importance with regard to material protection, the avoidance of toxic compounds potentially dangerous for the bacteria.

Bacteria use a communication system called *Quorum sensing*, using small molecule signals to moderate their behaviour. The involvement of an intercellular signal molecule in the development of biofilms suggests possible targets to control or prevent biofilm growth (Davies et al., 1998).

Several signalling molecules have been identified, but there is yet no product marketable.

3.7 New technologies in research

Finding the ideal active compound in material protection is as in pharmaceutical or agrochemical research like looking for a needle in a hay stack.

Thousands of compounds have to be synthesized and screened to find the best candidate. The discovery of new lead structures and their further optimization involves high risks and costs. Because of the enormously high follow up cost for toxicological studies, development and registration, wrong decisions have to be avoided.

Therefore new techniques are required which can help to reduce the time, cost or risk involved in R&D.

The introduction of new methods to accelerate the development was driven by R&D in life sciences. In the meantime, similar methods have come in use in the research for material protection as well as the research for new materials, polymers and catalysts.

The process of finding new active ingredients can generally be divided into two main steps:

- finding a new lead structure
- optimizing the lead structure into a development candidate.

In order to accelerate the finding of new lead structures, two separate methods are in use:

- rational drug design
- synthesis and screening of large random libraries.

In general, both methods can be used independently or together.

If the desired target is known and structural information of the enzyme or a complex of the enzyme with an inhibitor are available by x-ray diffraction, NMR or homology-modeling, the method of 3-dimensional molecular modeling is used to see if potential inhibitors are able to interact with the target site (Goodman, 1998). Binding of the potential inhibitor to the receptor may include hydrophobic, electrostatic or hydrogen-bonding interactions. In more advanced techniques, solvation energies of the ligand and the receptor site are considered because partial or complete desolvation must occur prior to binding.

In most cases however, the precise structure of the target is unknown and the ligand-based approach has to be applied. This approach can be used when a series of compounds have been identified that exert the activity of interest. To be used most effectively, one should have structural similar compounds with high activity, no activity and with a range of intermediate activities. In recognition site mapping, an attempt is made to identify a pharmacophore, which is a template derived from the structure of these compounds. The pharmacophore is represented as an arrangement of functional groups in three-dimensional space which is complementary to the geometry of the receptor side. As the pharmacokinetics of the actives are not considered, it is not possible to predict if a potential inhibitor will ever reach the site of interest.

Another way to overcome the relatively low chance to find a new lead structure is to increase the number of tested compounds by screening large libraries of diverse structures.

With the introduction of new high-throughput screening methods on isolated enzymes or intact micro-organism, together with an extreme low substance demand, the biological testing is not longer the eye of the needle.

Parallel synthesis in solution, which means the creation of a series of individual compounds through reactions performed simultaneously, rather than one at a time, was the first step to increase the number of compounds. The basic chemical procedures are usually similar to classical synthesis but performed with the aid of automatic systems (Brümmer et al., 1999). Such automatic systems are ranging from simple liquid-handler based workstations to fully automated robot systems which are able to perform all steps of synthesis, work-up, purification and aliquotation.

The majority of the compound libraries in the pharmaceutical research of the last years have been synthesized on a solid support (insoluble material to which the compounds are covalently attached during the synthesis) in automatic systems (Früchtel et al., 1996; Gordon et al., 1994). The main advantage of the solid-phase synthesis is that the solid support makes it easier to separate the products from the reactants, so the reactants can be used in excess to drive the reactions to completion.

During the optimization of a lead structure to a development candidate, all of the above mentioned methods can be used as well.

Additionally, QSAR (quantitative structure-activity relationship) is used in the optimization process (Seydel et al., 1979; Draber et al., 1992). QSAR represents an attempt to correlate structural or property descriptors of compounds with biological activities. The physicochemical descriptors include parameters to account for hydrophobicity, topology, electronic properties and steric effects. The method is also used to get informations how to decouple undesired toxic effect from desired biological activity.

3.8 Testing of new biocides

In order to find and develop new biocides, an exact knowledge of the relevant application fields and use conditions is important. Furthermore, the relevant materials destruent need to be known and testing methods have to be developed. Beside a good antimicrobial activity, new innovative compounds have to fulfil also a range of physico-chemical requirements. Modern biocides should have a low toxicity and promising ecotoxicological properties like for example a good biodegradability.

The introduction of a new antimicrobial compound is only possible if legal registration requirements can be met. However, these requirements differ in many European countries and also in other relevant markets like USA, Canada and Japan. In Europe, the biocidal products directive (BPD) will harmonize the registration of such products.

An efficient screening requires that in a short time a large number of active compounds can be checked for their use as preservatives. In such a screening cascade, short and simple tests are located at the beginning, whereas at the end more complicated and work intensive test systems are located. Fast early screening tests are enabling the chemical research group to stop or to modify a synthesis program which is not promising.

The first step in a screening cascade is normally the determination of the minimal inhibition concentrations against bacteria, fungi or algae.

3.8.1 Screening for new fungicides

A fungicide mainly used for the preservation of paint films should have a high activity against a broad range of ascomycota and deuteromycota, whereas a fungicide used as a real wood preservative should show excellent performance against basidiomycota. Therefore most screening systems have incorporated in the first step in addition to a more or less broad range of ascomycota and deuteromycota at least one wood destroying basidiomycota species.

For an ideal film fungicide, activity gaps should not exist. Further tests in the cascade are often tests in accordance to EN 152-1 (European Standard EN 152-1, 1988) and tests in dispersion paints based on polyvinylacetate, styrol-acrylate and acrylate. An ideal film fungicide should furthermore show no leaching, a good UV stability and activity against algae.

In the first screening steps for a new fungicide against basidiomycota, parts of fungal colonies are placed on nutrient agar in which test compounds in different concentrations have been incorporated. After an incubation time of several days, the radial growth of these colonies is compared to biocide-free controls.

If the results are promising, further examinations will be done in accordance to international standards. In EN 113 (European Standard EN 113, 1990) for example, the toxic effects of test compounds against wood destroying basidiomycetes like *Coniophora puteana*, *Gleophyllum trabeum*, *Poria placenta* and *Coriolus versicolor* are determined. In this test, wood blocks are impregnated with different concentrations of the test compounds. In order to get more realistic indications of the properties of the compounds, EN 113 is often combined with accelerated ageing tests of treated wood prior to biological testing like EN 73 (evaporative ageing procedure) or EN 84 (leaching procedure) (European Standard EN 73, 1988; European Standard EN 84, 1997).

3.8.2 Screening for new Antibacterials

For many technical applications like for example the preservation of water based paints or metal-working fluids, there is increasing demand in the market for a biocide with a high antibacterial activity and beneficial toxicological and ecotoxicological properties due to the fact that most of the used biocides are more or less strong sensitizers.

In the ideal case, such a bactericide should – beside an excellent activity against Gram-negative bacteria – also have antifungal properties. It should be water soluble and should not contain halogen in the molecule.

In most cases Gram-negative bacteria like for example *Pseudomonas aeruginosa* or *Pseudomonas fluorescens* are used for an antibacterial primary screening. Compared to Gram-positive bacteria, inhibition of these bacteria is more difficult due to the different cell walls of Gram-negative and Gram-positive bacteria.

If the efficacy of the examined compounds is sufficient, in the next step a broader range of bacteria and fungi like *Geotrichum candidum*, *Fusarium sp.* and *Rhodotorula rubra* will be examined. Further test systems in such a screening cascade are normally laboratory based methods like metal-working fluid tests, long time inoculation tests and in-can preservation tests. At this stage, also important physico-chemical parameters like water solubility and stability in alkaline media are generally determined.

3.8.3 Screening for new insecticides

Selected insecticides and termiticides can be used for the protection of wood, wood composites and plastic. Due to the relative small market size for such applications, companies usually have no special material protection screening for insecticides.

If the physico-chemical properties like stability and solubility as well as ectotoxicological and toxicological data justify further examinations, threshold values for the effectiveness of the product against wood-destroying insects are determined. Methods often used are EN 46 (European Standard EN 46, 1990), in which the preventive action against recently hatched larvae of *Hylotrupes bajulus* (Linnaeus) is determined, and EN 117 (European Standard EN 117, 1990). In the last mentioned test, toxic values against termites like *Heterotermes indicola* and *Reticulitermes santonensis* are established without and after ageing of impregnated wood.

Some recent approaches use synthetic analogues of juvenile hormones (Menn and Henrick, 1981; Staal, 1982) or growth regulators. However in most cases the recently used test methods are not suitable to show efficacy of such compounds.

3.8.4 Screening for new actives against marine antifouling

Structures that are continuously submerged in water like hulls of ships become encrusted with a variety of aquatic organisms such as *Balanus*, *Anomia*, *Enteromorpha*, *Hydroides* and others. The attachment of these organisms causes economic damage in various ways: attachment to the hulls of ships enhances fuel consumption and causes loss of profitable time because of the need to clean the hulls (Redfield and Hutchins, 1952; Scrip, 1984).

For the primary antifouling screening, a barnacle assay is used in most cases. For this test, mass reared cyprid larvae of barnacle species like for example *Balanus amphitrite* are injected in dishes containing solutions of test compounds in seawater. After one hour, the settlement inhibition in regard to biocide-free controls is determined.

Compounds with promising effects in this test system will be checked very often also for the inhibition of algae and other marine organisms (Houghton, 1984). Further steps will be incorporation in antifouling paints and raft tests.

3.8.5 High throughput screening

As it has been outlined before, the finding of new lead structures can be accelerated by the synthesis and screening of large random libraries, involving High Throughput Screening. At Bayer, in collaboration with the crop protection business unit, a high throughput screening with material destruent has been established. A large number of new compounds evolving from chemical libraries or synthesized by different research groups, are screened against relevant destruent of materials belonging to taxonomic groups like Gram-negative bacteria, Ascomycota, Deuteromycota and Basidiomycota. Lead structures evolving from this high throughput screening are then further optimized for application fields like technical preservation, film protection and protection of wood against basidiomycota.

3.9 Conclusion

Despite the increasing R&D and registration cost, a considerable number of new compounds have been introduced into the material protection market during the last 10–15 years.

It is obvious that a lot of new compounds – especially in the fungicidal and insecticidal area – are “spin-offs” from agrochemical research. Important examples are azole fungicides, pyrethroids, benzoylureas and chloronicotinyl insecticides.

In addition to new chemical entities, there are other interesting new approaches e.g. in antifouling or in using enzymes as material protection products.

New technologies from life sciences, like the synthesis and high throughput screening of large compound libraries, can help to increase R&D efficiency and to accelerate the finding of new lead structures.

In future, the increasing R&D and registration cost will make it necessary to focus the search for new compounds for material protection even more on larger market segments which can offer the required pay-back.

In Europe, the Biocidal Product Directive (BPD) will make it necessary to find substitution products for compounds no longer registered and supported under the BPD.

In addition to new compounds, combining existing actives to offer new formulations and products will increase.

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4 Legislative aspects

4.1 United States antimicrobial pesticide regulations

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4.1.1 Introduction: Pesticides beyond the agricultural application

In 1970 President Richard Nixon signed Reorganization Order Number Three [1] and established the Environmental Protection Agency with public concern about pesticides as a driving force. With this Order the EPA inherited from the Department of Interior and specifically from the United States Department of Agriculture (USDA) the primary responsibility for the regulation of pesticides in the United States. Until the enactment of the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) [2] in 1972 the Agencies' activities were heavily influenced by the personnel and functions that had been reassigned from the Department of Interior. FIFRA gave the EPA a new authority to regulate pesticides, and while the EPA has expanded their purview to almost all chemicals and environmental media, FIFRA has served as a basis for the regulation of chemicals in the USA.

Pesticides offer great economic and social benefits through the protection and preservation of materials, food and the prevention of diseases. Since pesticides are designed specifically to fight harmful or even dangerous life forms, and therefore are toxic to them, they may present hazards to the environment by their potential effect upon non-target organisms, including humans, particularly when misused. The need to balance these benefits against the risks presents a challenge to the EPA unlike other chemicals.

4.1.2 Master plan wanted

Central to FIFRA is the pesticide registration process. This process has evolved over the last three decades into a process so comprehensive and complex that it seems to continually outpace the EPA's ability to administer it. Continuous new mandates coupled with public pressure have led to

- testing requirements for which there are no protocols,
- diversion of resources to reregistration when registration can not be accomplished,
- tolerance re-evaluations when reregistration is not complete,
- a proliferation of Pesticide Registration (PR) Notices that should not, but seem to, have the force of law, and
- interagency jurisdictional squabbles that have caused review and approval slowdowns and redundancies.

Usually, adequate amounts of toxicology, environmental and fate data are considered to be a luxury for most chemicals, for pesticides it is commonplace. Even the most modest data requirements, such as for pesticides classified in the "low exposure indoor" use category, are greater than for any other class of chemicals, save prescriptive drugs. While access to adequate data might seem to all, except those who must pay to complete it, a good thing, knowing too much about a substance can also have unintended consequences. Registrants who have conducted chronic studies with equivocal results often find themselves competing against a chemical in the marketplace that has not been required to have a chronic study conducted. A pesticide assigned only to an "indoor" use category would not be required to provide the same level of data as one classified as a "food" use. This makes perfect sense until the "food" use chemical can also have application in a "non-food" or "indoor" category. Then the existence of additional data with equivocal endpoints can be used against the chemical, even though it may ultimately be safer than others in this category. Consequently additional safety factors are applied because of these uncertainties that distort relative risk.

Before going into the details of the registration process it should be noted that how efficiently a registration gets through the EPA process is dependent on the registrant's ability to properly procure the toxicological and environmental properties of the subject compound and convey them to the EPA in the mode requested. This is by far the most expensive and time consuming element, and although it is not the subject of this paper a few comments on experience with the process may be allowed.

Six acute toxicology studies (oral, dermal, inhalation, skin and eye irritation, dermal sensitization well known as "six-pack"), are necessary and few would argue that they can establish the hazard of a product for labeling purposes. The result places the product in one of four categories that will be further explained below in the labeling section of the registration requirements (see p. 8). Each formulation must undergo this battery of six studies whose results dictate which category applies and thus the severity of the warnings and practical treatment advice. What could be argued, however, is the EPA's inflexibility in bridging toxicology data from similar

formulations. Hazard warnings and practical treatment advice required by the four categories of hazard (poisonous, harmful, corrosive, irritant) are often indistinguishable, to the average user, yet formal tests must be run with little ability to extrapolate, even to a category which will in the end require the same personal protective equipment and require identical first aid.

One of the six acute studies is dermal sensitization. There are several types of sensitization studies that are acceptable and there is a footnote in the data requirements tables that allows for an exemption from the study if prolonged and repeated contact does not occur. All positive responses, regardless of the severity of the study, require a warning statement indicating sensitization and products without prolonged and repeated exposure may require no statement as the study may have been exempted. On one hand the EPA requires testing to split fine lines, but in the case of sensitization no distinction is made for severity.

Results from longer term studies, e.g. subacute or subchronic repeated dose applications over twenty-eight or ninety days respectively, determine target organs, various effect levels and are used to choose the dosage for chronic/carcinogenicity studies. Findings from a chronic toxicity study are used to determine the toxicity caused by long-term exposure to a compound and/or carcinogenicity. However, many of these studies have yielded results, that even in the same taxonomic category – most commonly used test animals are Sprague-Dawley rats, Fischer rats, Wistar rats and B6C3F1 mice – different toxicological findings may occur. To cope with this problem it is assumed by the regulatory agency that the toxic response observed in a particular species/strain will occur in humans also, unless a mechanism for the observed toxicity is known – especially the aspect of simple overflow of the metabolic capacity in the individual test animal should be taken into consideration – and it is common knowledge that this mechanism for the induction of toxicity will not occur in humans. Although a battery of mutagenicity tests are to be conducted, these tests do not necessarily rule out or indicate a carcinogenic potential of a test compound. In general negative results in mutagenicity studies do constitute a basis for a waiver of long-term studies, but positive results may indicate the necessity of them. Thus, carcinogenicity studies alone can establish or rule out a carcinogenic potential of a compound, regardless of whether it makes sense to transfer the results to humans or not.

The “no observable effect levels” (NOELs) from these studies are used by the EPA reviewers as reference dose to calculate margins of exposure (MOE) based on aggregate exposure information. MOEs of greater than 100 are generally considered adequate for registration. These calculations have however never taken into consideration cumulative factors such as exposure to compounds of similar toxic mechanism and the lifestyle of the person exposed. The FQPA has mandated the EPA to look at cumulative exposure and the EPA is currently dealing with how to accomplish this.

As will be explained in the following pages the evolution of the registration process has culminated in a split of registration responsibilities within the Agency into the Registration Division handling the registration of insecticides, herbicides, and plant fungicides and growth control chemicals, and the Antimicrobial Division handling industrial biocides such as preservatives, water treatment chemicals, antifoulants, and disinfectants. The split was actually mandated by the Food Quality Protection Act [3] (FQPA) and has been in place for approximately five years. While there are many positive aspects to this split there have also been some downsides.

Probably the most significant positive aspect of the institution of the Antimicrobial Division has been the development of revision to Section 158 of the Pesticide Code of Federal Regulations. This section specifies the data required for each use category. As will be pointed out in more detail later these categories were tailored for agricultural application and have made determining industrial biocide data requirements difficult. While the proposed Section 158 revisions probably increase the amount of data required, it does at least allow a more realistic up-front determination of the potential registration cost and timing. But again, this action has been in “proposed” form for approximately seven years, leaving registrants to wonder which requirements apply. The EPA seems to be able to use the action as if it were law to deny registrations or require additional data.

4.1.3 The enigma of classification

Take, for instance, the toxicological endpoint of cancer. The EPA’s 1986 “Guidelines for Carcinogenic Risk Assessment” [4] provides guidance on how to assess carcinogenic risk. After going through a laborious evaluation the resulting classification scheme is as follows.

Group A: Human carcinogen. This group is used only when there is sufficient evidence from epidemiologic studies to support a causal association between exposure to the agents and cancer.

Group B: Probable human carcinogen. This group includes agents for which the weight of the evidence of human carcinogenicity based on epidemiologic studies is “limited” and also includes agents for which the weight of the evidence of carcinogenicity based on animal studies is “sufficient.” The group is divided into two subgroups. Usually, Group B1 is reserved for agents for which there is limited evidence from epidemiologic

studies. It is reasonable, for practical purposes, to regard an agent for which there is “sufficient” evidence of carcinogenicity in animals as if it presented a carcinogenicity risk to humans. Therefore, agents for which there is “sufficient” evidence from animal studies but for which there is “inadequate evidence” or “no data” from epidemiologic studies would usually be categorized under Group B2.

Group C: Possible human carcinogen. This group is used for agents with limited evidence of carcinogenicity in animals in the absence of human data. It includes a wide variety of evidence, e.g.,

- (a) a malignant tumor response in a single well-conducted experiment that does not meet conditions for sufficient evidence,
- (b) tumor responses of marginal statistical significance in studies having inadequate design or reporting,
- (c) benign but not malignant tumors with an agent showing no response in a variety of short-term tests for mutagenicity, and
- (d) responses of marginal statistical significance in a tissue known to have a high or variable background rate.

Group D: Not classifiable as to human carcinogenicity. This group is used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.

Group E: Evidence of non-carcinogenicity for humans. This group is used for agents that show no evidence for carcinogenicity in at least two animal tests in different species or in both adequate epidemiologic and animal studies.

The designation of an agent as being in Group E is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

The comments and problems with this oversimplified classification system were immediately recognized by stakeholders and the draft was never finalized. It was however used by the EPA in the review of pesticides. In two subsequent drafts other types of designations are proposed, however these have not been finalized. The new classifications are:

Known/Likely: This category of descriptors is appropriate when the available tumor effects and other key data are adequate to convincingly demonstrate carcinogenic potential for humans; it includes:

Agents known to be carcinogenic in humans based on either epidemiologic evidence or a combination of epidemiologic and experimental evidence, demonstrating causality between human exposure and cancer.

Agents that should be treated as if they were known human carcinogens, based on a combination of epidemiologic data showing a plausible causal association (not demonstrating it definitively) and strong experimental evidence.

Agents that are likely to produce cancer in humans due to the production or anticipated production of tumors by modes of action that are relevant or assumed to be relevant to human carcinogenicity.

Modifying descriptors for particularly high or low ranking in the “known/likely” group can be applied, based on scientific judgment and experience and are as follows:

Agents that are likely to produce cancer in humans based on data that are at the high end of the weights of evidence typical of this group,

Agents that are likely to produce cancer in humans based on data that are at the low end of the weights of evidence typical of this group.

Cannot be determined: This category of descriptors is appropriate when available tumor effects or other key data are suggestive or conflicting or limited in quantity and, thus, are not adequate to convincingly demonstrate carcinogenic potential for humans. In general, further agent specific and generic research and testing are needed to be able to describe human carcinogenic potential. The descriptor cannot be determined is used with a subdescriptor that captures the rationale:

Agents whose carcinogenic potential cannot be determined, but for which there is suggestive evidence that raises concern for carcinogenic effects,

Agents whose carcinogenic potential cannot be determined because the existing evidence is composed of conflicting data (e.g., some evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm any concern), agents whose carcinogenic potential cannot be determined because there are inadequate data to perform an assessment,

Agents whose carcinogenic potential cannot be determined because no data are available to perform an assessment.

Not likely: This is the appropriate descriptor when experimental evidence is satisfactory for deciding that there is no basis for human hazard concern, as follows (in the absence of human data suggesting a potential for cancer effects):

Agents “not likely” to be carcinogenic to humans because they have been evaluated in at least two well conducted studies in two appropriate animal species without demonstrating carcinogenic effects,

Agents “not likely” to be carcinogenic to humans because they have been appropriately evaluated in animals and show only carcinogenic effects that have been shown not to be relevant to humans (e.g., showing only effects in the male rat kidney due to accumulation of alpha2u-globulin),

Agents “not likely” to be carcinogenic to humans when carcinogenicity is dose or route dependent or for instance, not likely below a certain dose range (categorized as likely by another route of exposure). To qualify, agents will have been appropriately evaluated in animal studies and the only effects show a dose range or route limitation or a route limitation is otherwise shown by empirical data,

Agents “not likely” to be carcinogenic to humans based on extensive human experience that demonstrates lack of effect (e.g., phenobarbital).

In a third draft, not yet published yet another classification scheme is proposed.

Carcinogenic to humans: This descriptor is appropriate when there is convincing epidemiologic evidence demonstrating causality between human exposure and cancer. This descriptor is also appropriate when there is an absence of conclusive epidemiologic evidence to clearly establish a cause and effect relationship between human exposure and cancer, but there is compelling evidence of carcinogenicity in animals and mechanistic information in animals and humans demonstrating similar mode(s) of carcinogenic action. It is used when all of the following conditions are met:

There is evidence in a human population(s) of association of exposure to the agent with cancer, but not enough to show a causal association, and

There is extensive evidence of carcinogenicity, and

The mode(s) of carcinogenic action and associated key events have been identified in animals, and

The key events that precede the cancer response in animals have been observed in the human population(s) that also shows evidence of an association of exposure to the agent with cancer.

Likely to be carcinogenic to humans: This descriptor is appropriate when the available tumor effects and other key data are adequate to demonstrate carcinogenic potential to humans. Adequate data are within a spectrum. At one end is evidence for an association between human exposure to the agent and cancer and strong experimental evidence of carcinogenicity in animals; at the other, with no human data, the weight of experimental evidence shows animal carcinogenicity by a mode or modes of action that are relevant or assumed to be relevant to humans.

Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential: This descriptor is appropriate when the evidence from human or animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is judged not sufficient for a conclusion as to human carcinogenic potential. Examples of such evidence may include: a marginal increase in tumors that may be exposure-related, or evidence is observed only in a single study, or the only evidence is limited to certain high background tumors in one sex of one species. Dose-response assessment is not indicated for these agents. Further studies would be needed to determine human carcinogenic potential.

Data are inadequate for an assessment of human carcinogenic potential: This descriptor is used when available data are judged inadequate to perform an assessment. This includes a case when there is a lack of pertinent or useful data or when existing evidence is conflicting, e.g., some evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm a concern.

Not Likely to be carcinogenic to humans: This descriptor is used when the available data are considered robust for deciding that there is no basis for human hazard concern. The judgment may be used on:

Extensive human experience that demonstrates lack of carcinogenic effect (e.g., phenobarbital);

Animal evidence that demonstrates lack of carcinogenic effect in at least two well-designed and well-conducted studies in two appropriate animal species (in the absence of human data suggesting a potential for cancer effects);

Extensive experimental evidence showing that the only carcinogenic effects observed in animals are not considered relevant to humans (e.g., showing only effects in the male rat kidney due to accumulation of alpha2u-globulin);

Evidence that carcinogenic effects are not likely by a particular route of exposure;
 Evidence that carcinogenic effects are not anticipated below a defined dose range.

As these various categorizations indicate the EPA establishes a regulation which has a particular unintended effect and attempts to clarify and then never finalizes the clarification. This puts the classification process in a chaotic state that created a bias against chemicals depending on when they went through the process. Unless they are also companies that have had to deal with the EPA registration process, end users often do not understand these distinctions.

4.1.4 A dialogue with a registrant

In the following pages the responses to a series of very pointed questions will present a comprehensive review of the FIFRA registration process. It should be remembered that the registration process is a continuously evolving process and data or data waivers that were acceptable for one review period, may not be for another. The Antimicrobial Division has made some progress in leveling the playing field in terms of data consistency and adherence to review time mandate, but there is still much work to be done.

—*What caused the authorities initiative?* While federal regulation of pesticides began with the Insecticide Act of 1910 [5] pesticide regulations have been in their current form since the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) was amended in 1972. This amendment, the Federal Environmental Pest Control Act of 1972 transformed FIFRA from a registration process that prohibited misbranding to a process that looked at “unreasonable” effects on human health and the environment. It also gave the Environmental Protection Agency (EPA) broader authority to regulate pesticide use and to reregister all previously registered pesticides using up-to-date standards. The initiative for such broad change in what is required for registration came mainly as a result of the increasing number of products that resulted from the initial success of DDT [6].

Having made little progress in the reregistrations of these products by 1988, the EPA’s hand was forced by Congress, reacting to public pressure, when they legislated an amendment to FIFRA [7] mandating a specific five-phase program to bring all existing registrations up to modern standards. Public concern was caused over events such as a kepone spill, links of specific chemicals to specific types of diseases, awareness of the persistence in the environment of chemicals such as pentachlorophenol (PCP) and polychlorinated biphenyls (PCBs). This amendment required that every product registered prior to 1984 be reregistered. This process is ongoing, despite the fact that mandated completion dates have come and gone, and reregistration fees of \$150,000 for each active were collected to provide additional resources for the task. Each registrant is being required to repeat expensive studies that were not run according to Good Laboratory Practice (GLP) [8].

By subjecting older registrations to these requirements and imposing these requirements on new submissions, eventually all registrations would be brought up to current standards. In reality this process has forced those manufacturers that could not afford to conduct the additional testing to abandon these products confining the registration of actives with a small number of suppliers. This is particularly true in the antimicrobial area.

Finally in 1996 the Food Quality Protection Act (FQPA) was passed with stringent new cumulative risk assessment requirements for chemicals regulated by the EPA, through FIFRA, and the FDA, through the Federal Food Drug and Cosmetics Act (FFDCA). An additional unintended consequence of this Act has been the blurring of the jurisdictional line between the EPA and FDA when it comes to the review of pesticides that are also indirect food additives. The EPA currently believes that the FQPA grants them the authority to conduct FDA-style reviews on antimicrobial, indirect food additives using their own standards even though the FDA has already approved the product for the application. This is particularly unfortunate in that the FDA has recently initiated the Food Contact Notification (FCN) process that has greatly improved their handling and approval rate of indirect food contact substances. So while the FDA process has become more certain, the EPA review process of these chemicals has become much less so.

—*Why is registration mandatory?* Antimicrobial pesticides are brought into the purview of FIFRA by its definition of “pest”. A pest, according to FIFRA §2(u) is:

“... (1) any insect rodent nematode, fungus, weed, or (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest”

A pesticide then is:

“... any substance or mixture of substances intended: (a) for preventing, destroying, repelling or mitigating any pest ...”

A chemical or mixture is not a pesticide until it is used for the above purposes and it cannot be used for such purposes until the EPA determines through the registration process that there are no unreasonable effects to human health or the environment.

—*By what law is an active substance or its preparation regulated?* If a chemical is being used according to the definition of a pesticide above, it is regulated by FIFRA. To be approved by FIFRA the manufacturer must satisfy the many requirements of FIFRA. What specific requirements must be fulfilled depends on the application or “end-use”. If a material’s use pattern, or use claims, are only to sell to formulators in order to formulate products that will themselves need registration, the material will be registered as a “technical grade active ingredient” (TGAI) or “manufacturing-use product” (MUP).

If the material is to be sold directly to the end user, the material will have to meet all the requirements of the use patterns that are claimed on the label and be registered as an “end-use product” (EP). If most of the requirements for the active ingredient have been met for the TGAI by a previous registrant, the registrant of the EP can take advantage of the existing data. There are several ways that this can be done.

- If the manufacturer of the MUP also has an EP registered that is identical, a supplemental, or sub-registration may be sought from a registrant provided that the uses are also identical.
- If the EP registration applicant is purchasing a registered MUP directly from the registrant, the EP registrant is exempt from providing any data that the MUP registrant has provided because the cost of the data is reflected in the price of the MUP.
- If the EP registrant wishes to have a different unregistered source of the TGAI, all of the required data must be submitted for the TGAI by either re-conducting the studies or offering to pay the registrant who does have a registration.

MUPs have all of the very rigorous toxicology data but not many exposure and environmental impact related requirements. This is because their use is primarily in the plant formulating the EP which itself must undergo registration. It is during the registration of the EP that the registrant must address potential exposure and environmental impact.

To use as an example an antifoulant coating the TGAI must first be registered. To accomplish this, the manufacturer must provide all of the short and long-term toxicology associated with the active and a release study that shows the potential release of the active into water ways when incorporated into a typical coating. This release study has an effect on how and if environmental fate studies are required. The coating itself would then have to be registered, because the claim made encompasses something beyond the coating itself. In other words the coating prevents marine “pests” from attaching to the boat. Each coating formulation, color or other variation would have to obtain its own registration. These coating registrations require only acute toxicology, but concentrate heavily on actual exposure assessments for each use claimed. Formulating these coatings with other ingredients (inerts) previously known to the EPA [9] will greatly shorten the review time.

—*What is authorized by the EPA?* In 40 CFR 158, there are tables of data requirements that are split into use categories. These use categories have been very broad and difficult to use over the years. This has prompted data-call-ins to require additional data based on certain use categories. The 1987 Antimicrobial Data Call-In, for instance, required data to be submitted for certain uses with higher exposure characteristics and therefore additional data requirements that could be ascertained from strict adherence to the regulatory tables.

This section of the regulation does however serve as a guide for data requirements. For instance, in the example of antifoulant paints, the use falls into the category of “aquatic non-crop”. From this, the tables can be used to determine a set of requirements. Though often not straightforward a tedious navigation through all of the conditional requirements with accompanying footnotes can bring a registrant very near to a core set of data required for a product that can make such claims on its label.

Antimicrobial pesticides generally fall into the “indoor” use category since this is the set of data requirements that most closely approximates the type of exposure associated with their use. The antimicrobial registrants have always felt that these categories were better suited to the agricultural applications and have worked with the EPA to develop separate antimicrobial data tables that provide requirements to better assess the risk of these products. There has been progress on this since the enactment of the Food Quality Protection Act (FQPA). The Act has prompted the establishment of the Antimicrobial Division, and new drafts of regulations in 40CFR158 that will establish antimicrobial specific use categories and much better defined requirements. The twelve new categories are as follows:

- Agricultural
- Food Handling
- Commercial/Institutional/Industrial
- Residential and Public Access
- Medical Premise

Human Drinking Water
 Material Preservation
 Industrial Processes and Water Systems
 Antifoulant Coatings
 Wood Preservation
 Swimming Pools
 Aquatic Areas

Whereas previously there had been eleven categories to cover all pesticides, there will now be twelve categories just to cover antimicrobials, making data requirements much more straightforward. These may change in the final rule but for now these categories will guide the requirements for antimicrobials.

—*In what form has one to submit the data set to the relevant authority?* With the diversity of registration types, chemicals, uses, claims and the extensive data required to support them, a registration can range from a few pages to many volumes. Some general statements can be made concerning exactly what need to be contained in an antimicrobial registration application. An application should address the following areas:

Administrative materials

- *A transmittal document* – This is basically a cover letter listing each component of the submission. This document is copied and included as the cover to each separately bound volume in the submission.
- *A registration application form (EPA# 8570-1)* – This form should be included in every registration. This is a simple form identifying the submitter, the product trade name, the packaging, the registration type, and a short explanation as to why the submission is being made.
- *A Confidential Statement of Formula (EPA# 8570-4)* – This form is required for all registrations that are not supplemental registrations (re-labeling of an already registered product) or amendments to the label of an already registered product. This form very specifically identifies the ingredients and their function, upper and lower limits, source and nominal concentration of each. There is a CSF on file for each registered pesticide.
- *Formulator's Exemption Statement (EPA# 8570-27)* – This form applies for an exemption from generic data requirements based on the registration of the source active. If the active ingredient in the registration application is from a registered source, the applicant is exempt from providing any of the data already resident at the EPA to support the source product.
- *Certification with Respect to Citations of Data (EPA# 8570-34)* – This form is required when any existing data is being cited in the registration application. This is a signed certification that the submitter has taken the proper steps to compensate, to the extent required by FIFRA 3(c)(1)(F) 3(c)(2)(B), the owner(s) of the data being cited. In this way the EPA is assured that proper compensation negotiations have been initiated and will reach a conclusion separate from the registration process.
- *Data Matrix (EPA# 8570-35)* – This form is a listing of the data that the submitter has identified as being required for the registration, its source, guideline requirement number and its "status". This form is used for new registrations, amendments with uses requiring new additional data where the "selective" method of supporting data is employed. The selective method cites specifically identified studies that are new, in the literature or that have previously been submitted by the applicant or a former applicant. The "status" response for this form gives the following options:

- OWN – I am the Original Data Submitter for this study
- EXC – I have obtained written permission to cite this exclusive-use study
- PER – I have obtained the permission of the Original Data Submitter to use this study
- OLD – The study was submitted more than 15 years ago and all compensation periods have expired.
- PL – The study is in the public literature
- PAY – I have notified in writing the Original Data Submitter or, if the cite-all method is used, all companies listed in the Data Submitter List for this ingredient, and have offered to
 - (a) pay compensation in accordance with FIFRA 3(c)(1)(F) 3(c)(2)(B), or
 - (b) to commence negotiation to determine the amount and terms of compensation, if any, to be paid for the use of the study(ies).
- GAP – This guideline requirement is a data gap as defined by 40 CFR section 152.83(a) and 152.96.
- FOR – I am taking the formulator exemption for this ingredient only.

There is also a public information version of this form that must be completed that keeps the studies and reference numbers confidential. Also if the submitter chooses to use the "cite-all" method of data this form becomes unnecessary, because all data resident at the EPA is being cited and all data submitters will have been informed by letter and certified using the previously mentioned form, EPA 8570-34.

There are also other forms for specific types of action such as Experimental Use Permits (EUP) and Special Local Needs [24 (c)] (SLN), but the standard registration application will have included the above administrative materials.

– *Labeling*—Six copies of the products labeling are required. The EPA has published a “Label Review Manual” [10] which has helped in removing some of the subjectivity of the reviewers, however there are still many inconsistencies in the application of the standard. In general the manual has four hazard categories. Based on the toxicology results for the so-called “six-pack”, a category is assigned for each route of exposure. From category I, the most severe to the least severe, category IV, hazard warnings are chosen for each route of exposure to construct the “hazards to humans and domestic animals” section of the labeling. The “six-pack” study results determine the category and the hazard; personal protective equipment and first aid statements are determined by the category.

It is also important to remember that the EPA distinguishes between the product “label” and product “labeling”. The label being the portion of the labeling that actually gets attached to the product container, and the labeling is any additional document to which the label refers. The EPA must review all of these documents. One of the six copies will be stamped by the EPA and returned to the applicant signifying product approval.

Product chemistry. The product chemistry submission will contain a section identifying the chemical, a methodology for analysis, the manufacturing process, and identification of the raw materials and a discussion about the impurities present. A second section will give physical chemical properties of the product. The specific requirements will depend on whether the product is a manufacturing use product (MUP) or a technical grade active ingredient (TGAI). There are, of course, opportunities to waive data requirements that do not apply, but each must be addressed formally in the submission.

Certain chemistry studies are required to be conducted by EPA Good Laboratory Practice (GLP) and some require only substantial adherence to the EPA Chemistry Guidelines. Those chemistry endpoints subject to GLP are:

- Solubility
- Octanol/water partition coefficient
- Stability
- Volatility
- Persistence (such as biodegradation, photodegradation and chemical degradation)

New EPA guidelines allow that the applicant “self-certify” the physical/chemical properties data. There are forms for this (EPA 8570-36 and 37) which require the applicant to certify that the testing results are reliable and in “substantial conformity” with EPA guidelines. The EPA reserves the right to request any and all supporting data. [11]

Toxicology. Determining the exact toxicology requirements for a particular submission is probably the most difficult part of the exercise. They are dependent on the type of application, the intended use, likely exposure and what labeling restrictions the submitter is willing to accept. Antimicrobial product registration applicants have always had the additional difficulty of trying to determine what testing was required from a set of regulations that were developed with agricultural pesticides in mind.

In order to begin looking at the requirements for toxicology the applicant must first determine into which category the product’s end uses fall. The nine categories for “General Use Patterns” are:

- Terrestrial Food crop
- Terrestrial Nonfood
- Aquatic Food crop
- Aquatic Nonfood
- Greenhouse Food crop
- Greenhouse Nonfood
- Forestry
- Domestic outdoors
- Indoor

Antimicrobial products were usually put into the “indoor” use category and subject to a generic set of requirements, regardless of the exposures associated with that use.

This problem was addressed in the EPA 1987 Antimicrobial Data-Call-In [12] (DCI) that further categorized antimicrobial uses into high, medium and low exposure-types and set up a tiered data requirement. This

approach was carried into the reregistration process when FIFRA was amended in 1988. A higher level of toxicology was, of course, required for a higher level of exposure.

- In this DCI high exposure data requirements were imposed on swimming pool products, human drinking water treatments, food and non-food contact surfaces, hospitals and other health care facilities, metalworking fluids, laundry and dry cleaning chemicals and textiles for clothing or furnishing.
- To the medium category was assigned, industrial water processing systems, air washers and cooling towers.
- Low exposure uses were identified as adhesives, paperboard, paints, coatings, non-contact textile, leather and actives to treat lakes, ponds, sewage systems and secondary oil recovery systems.

While this DCI clarified the requirements for antimicrobial registrations, it imposed additional toxicology on high exposure categories that was previously not required by a strict reading of the regulations.

The DCI did allow mitigation of these requirements by providing exposure data that would indicate a lack of exposure significant enough to merit chronic testing. In response to this the American Chemistry Council (ACC), formerly the Chemical Manufacturer's Association, facilitated a group of antimicrobial registrants in conducting an exposure study [13] which evaluated actual antimicrobial exposure in a variety of applications. This study was submitted to the EPA in response to the requirements of the DCI and sponsors were able to waive much of the chronic data that would have been required.

Thus, to determine the toxicology requirements for an antimicrobial pesticide registration application the submitter must first make a determination of use category using 40 CFR 158, Appendix to Part A. This gives a listing of uses and the category for each. This category will be one of the above nine and will specify a generic list of requirements and conditional requirements. After a careful analysis of the list, its conditions and potential opportunities to waive a particular requirement for a particular use, a set of requirements can be developed. This list can be compared to the antimicrobial DCI to determine if additional requirements are added by the DCI for the application.

The data requirement tables also specify if the data point is to be run on the TGAI, on the end use product and in some cases on the pure active ingredient. This must be taken into account when trying to determine registration requirements. If for example you are registering a formulation, such as an antifouling paint, the requirement to conduct, for instance sub-chronic studies will be imposed on the TGAI not on the formulation itself. This TGAI data must either be provided or cited (with appropriate compensation) by the paint registration applicant.

It is always advisable to take the conclusions reached by this exercise to the EPA for a pre-registration meeting. They are always willing to meet with applicants to review the conclusions and discuss possible waivers. Timing on these meetings is often very critical however, they should be close enough to the submission so that the requirements have not changed, and yet far enough away to conduct any study that may be required that had not been anticipated.

Environmental toxicity and environmental fate. While antimicrobial products do not present significant environmental and fate concerns in most cases, there are several applications that do: once-through cooling towers, antifoulant coatings, and various treatments for swimming pools and other water systems. The process is the same as determining the toxicology required and for many antimicrobial products the result will be simply a hydrolysis study. Additional information may be required if the toxicity profile of the degraded components are of concern.

All pesticide labeling does address environmental concerns with the following statements and defers some of the environmental monitoring to the EPA regional offices through the NPDES Permitting process. [14]

Environmental hazards. This pesticide is toxic to estuarine invertebrates. Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to the discharge. Do not discharge effluent containing this product into sewer systems without previously notifying the sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA.

The first sentence in this paragraph can be changed based on results of environmental testing for fish, algae or invertebrates or may be completely unnecessary. The remainder is standard language that is required on all pesticides except in some cases where small quantities may be exempted. [15]

Exposure. With the establishment of cumulative risk assessment requirements of the FQPA, there is much more of a need to focus on exposure. Prior to FQPA registrants could often live with the worst case result of an individual exposure assessment. Hazards could be mitigated with additional testing and/or label recommendations for additional personal protective equipment. Cumulative assessment and a certain total allowable risk shows that worst-case exposure analyses soon cause the "risk cup" to overflow. Prudent long-term strategy for a

multi-use active ingredient will focus heavily on determining sound exposure assessments. Currently the EPA Antimicrobial Division has no real requirement for quantitative exposure assessment.

Antimicrobial exposures tend to be so much lower than agricultural applications. What is becoming apparent is that registrants of dual use pesticides, or pesticides that have applications registered in both the Registration and Antimicrobial Divisions, need to be extremely careful of the risk contribution of additional applications that are added to a risk cup that may already be somewhat filled. In other words a product registered as a plant fungicide may have value in an Antimicrobial Division regulated application such as wood preservation. The registrants must consider aggregate exposure from all uses in determining the viability of the product.

—*Is there a model dossier available?* The nearest thing that the EPA has to a model registration package is included in a registration “kit” [16]. This can be accessed on line at the following address (<http://www.epa.gov/pesticides/registrationkit/>) or can be ordered by mail. This publication explains the types of registrations possible, provides examples of the necessary forms and gives several examples of how a submission should be formatted. It does not go into detail on how to determine data requirements.

Also very helpful in determining labeling requirements for registration applications is the previously mentioned Label Review Manual. This manual has helped to level the playing field in requirements for labeling. The registration process has a natural bias toward new materials where the most stringent labeling requirements are imposed on the products that have most recently gone through the process. While this manual does not entirely solve the problem it does impose consistent requirements on new registrants that should be equally applied in the reregistration process. It also has made the label review process much more transparent.

—*Is there a chance of getting a preliminary registration?* It is unusual in the Antimicrobial Division of the EPA to obtain an “unconditional” registration. Registrations are usually issued on a conditional basis. This allows the EPA certain leeway in requesting additional data or, if necessary, instituting cancellation proceedings. In the Registration Division (Agricultural Review Division) they have been using a process called “time-limited” registrations. This is a registration that is issued on a short-term basis while certain data is being developed.

This practice has filtered into the Antimicrobial Division and has caused concern for some producers. They feel that there is no statutory support for this process and that the registration may be cancelled without proceeding, should the data be unavailable in the agreed time period. Other producers are willing to take the risk to gain earlier market entry.

—*Are there any exemptions to costly procedures?*

– *For research and development activities?* In 40 CFR 152.15 the EPA describes three things that are necessary for a product before it requires registration. The text of this regulation reads as follows:

“... A pesticide is any substance (or mixture of substances) intended for a pesticidal purpose, i.e., use for the purpose of preventing, destroying, repelling, or mitigating any pest or use as a plant regulator, defoliant, or desiccant. A substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide requiring registration, if:

- (a) The person who distributes or sells the substance claims, states, or implies (by labeling or otherwise):
 - (1) That the substance (either by itself or in combination with any other substance) can or should be used as a pesticide; or
 - (2) That the substance consists of or contains an active ingredient and that it can be used to manufacture a pesticide; or
- (b) The substance consists of or contains one or more active ingredients and has no significant commercially valuable use as distributed or sold other than
 - (1) use for pesticidal purpose (by itself or in combination with any other substance),
 - (2) use for manufacture of a pesticide; or
- (c) The person who distributes or sells the substance has actual or constructive knowledge that the substance will be used, or is intended to be used, for a pesticidal purpose.”

Thus if the substance in question is being used for research and development purposes, it does not meet the test of being used for a “pesticidal purpose” and would not need registration prior to initiating the research and development work.

This scenario even provides for limited field testing, provided that it remains within certain limitations. Exceeding certain limitation would require that an Experimental Use Permit (EUP) be obtained [17]. The EUP limitations are exclusively established in terms of agricultural uses, however, and it has historically been difficult to determine if an antimicrobial trial is within these limits.

When a registration is being prepared and data requirements are being determined, it is very important to pay close attention to the footnotes the EPA has included into 40CFR158. In these footnotes the EPA has listed a number of opportunities to apply for a waiver for certain requirements. Several examples are:

- an exemption for dermal toxicity, dermal and eye irritation if the pH of a material is less than two or greater than eleven and one half;
- an exemption from sensitization if dermal exposure is not expected to occur;
- an exemption from acute inhalation if, the condition of use, will not result in an inhalable material (e.g. gas, volatile substances, or aerosol/particulates);
- an exemption from sub-chronic and chronic studies if certain exposure criteria are met.

So even though there may be no exemption for the registration process itself, there may be an exemption for a specific data requirement.

It is also important to realize that there is no situation that will leave an activity unregulated. If the research and development activity does not meet the test to require registration under FIFRA, it is then covered by The Toxic Substances Control Act (TSCA) [18]. The relationship of TSCA and FIFRA is discussed in more detail in a subsequent chapter.

– *For only low volume sales and applications?* There are no exemptions to the pesticide regulations based on low volumes or sales in the Antimicrobial Division. In the Registration Division, however the term “minor use” is very significant. It is very difficult for low volume products to support the data required to obtain registration and remain on the market. Realizing the extensive data requirements for agricultural pesticides, particularly under the FQPA and the hundreds of minor use crops, Congress implemented special provision in the FQPA [19] to require the EPA to consult with growers prior to taking any action that might affect such products.

Because there are several hundred minor use crops, the easiest way to distinguish between them and major crops is to list the major crops. Major crops include almonds, apples, barley, beans (dry and snap), canola, corn (sweet and field), cotton, grapes, hay (alfalfa and other), oats, oranges, peanuts, pecans, popcorn, potatoes, rice, rye, sorghum, soybeans, sugar beets, sugarcane, sunflowers, tobacco, tomatoes, turf, and wheat.

In general the term applies to products that are applied to crops grown on less than a total of 300,000 acres. It also applies to products that are applied to major use crops that do not provide economic returns sufficient to cover registration costs. The United States Department of Agriculture (USDA) works with the EPA and State to ensure that important minor use products are supported. Obviously providers of pesticides for agricultural applications are far better off than those supplying actives for the material protection sector.

—*Are there any expedited registration processes?* FIFRA establishes expedited review procedures for a variety of Agency activities associated with the registration of pesticides, including expedited review for certain end-use pesticides that are identical or substantially similar to currently registered pesticides (“me too” registrations) as provided for in FIFRA section 3(c)(3)(B)(II), and for antimicrobial pesticides as provided for in FIFRA Section 3(h)(2).

FIFRA section 3(c)(10) establishes an expedited review for applications for registration and amendments to registrations for pesticides that “may reasonably be expected to accomplish one or more of the following:

- i. Reduce the risks of pesticides to human health.
- ii. Reduce the risks of pesticides to nontarget organisms.
- iii. Reduce the potential for contamination of groundwater, surface water or other valued environmental resources.
- iv. Broaden the adoption of integrated pest management strategies, or make such strategies more available or more effective.”

The statute does not establish deadlines for review of registration applications or amendments that meet the above criteria. Section 3(c)(10), however, requires EPA to notify the applicant whether the application for expedited review is complete not later than 30 days after receipt of the application.

The Food Quality Protection Act (FQPA) requires the EPA to develop procedures and guidelines for expedited review of any pesticide. These procedures and guidelines were outlined in a Pesticide Registration Notice [20]. [This PR Notice supersedes the reduced-risk criteria published in Federal Register Notices 57 FR 32140, July 20, 1992 and 58 FR 5854, January 22, 1993 and PR Notice 93-9, July 21, 1993]. The stated goal of the Reduced-Risk Pesticide Initiative

“is to encourage the development, registration and use of lower-risk pesticide products which would result in reduced risks to human health and the environment when compared to existing alternatives. The major incentive which EPA offers for these pesticides is expedited registration review. The major goal of the Antimicrobial Division is to provide expedited review of all types of antimicrobial registration actions.”

Aside from the “me too” registrations, none of these procedures have been very effective in expediting antimicrobial registrations. The difference in relative risks of these types of products makes opting for this type of review unattractive. The amount of upfront comparative work that is required, the difficulty of obtaining documented hazard information on competing products, and likelihood of an EPA acceptance of your conclusions more often make conventional registrations the better course.

Two recent activities may cause registrants to take another look at these guidelines. There has been a voluntary agreement by treated wood producers to cancel certain registrations for copper, chrome arsenate products [21 and in November 1999, the International Maritime Organization (IMO) Assembly adopted a resolution calling for a legally binding treaty prohibiting biocidal organotin compounds in antifouling systems on ship hulls by January 1, 2008. The EPA may be much more willing to work with registrants on reduced risk submissions.

—*Are there any regulatory negotiation processes?* There are several negotiation processes that may take place during the registration of an antimicrobial in the United States. There is the conditional registration. The Antimicrobial Division has diminished the effectiveness and meaning of the conditional registration by issuing all registrations as conditional. The condition imposed is usually that the registrants comply with any future data requirement imposed by the reregistration process. This gives the EPA more flexibility in recall and cancellation proceedings.

More recently, the EPA has been using “time-limited” registration to allow a certain negotiation process that was originally intended for the conditional registration. In the issuance of a time-limited registration a specific time is negotiated between the EPA and the registrant to complete some outstanding data requirement. If the data is not received in the specified time the registration automatically expires. It takes an affirmative action on the part of the EPA to allow the registration to continue. This gives the EPA even more control over the cancellation process, as they need to do nothing to allow it to expire.

Theoretically the time-limited registration allows the registrant to place a material on the market while a long-term study is being completed. The limited time would not allow exposures to get to chronic proportion until the results of the studies were known. Registrant companies are somewhat split on the use of such registration. Also, they are not specifically allowed by statute.

Perhaps the most useful negotiation process in antimicrobial registration is the negotiation of data waivers. There are a number of certain waivers as described previously, but if the registrant can construct a label to limit certain applications and thus limit certain exposures additional waivers may be possible. Waivers may also be possible if the exposure associated with a particular application can be limited with personal protection equipment recommendations to assure adequate protection against a certain kind of exposure. As an example, there are several expensive environmental fate requirements that are necessary for wood preservatives. If the registrant is willing to limit the use of the product to above ground use, these fate studies can be waived.

—*When and where do I have to contact a State EPA?* While the EPA has enforcement authority under FIFRA it also has the authority to grant states primary responsibility for enforcement. States must have an EPA approved enforcement program and they may enforce FIFRA. Generally, registered sites are visited by the State enforcement agency once a year and samples are taken to validate that the amount of active is within the allowed limits and that production records match annual production reports.

In addition States may grant a “Special Local Needs” (SLN) registration under FIFRA. This is a temporary registration granted to solve an immediate problem, for which there is no product registered that would provide a solution. These registrations are authorized under Section 24 (c) of FIFRA as follows:

“(1) A State may provide registration for additional uses of federally registered pesticides formulated for distribution and use *within that State to meet special local needs in accord with the purposes of this Act and if registration for such use has not previously been denied, disapproved, or canceled by the Administrator. Such registration shall be deemed registration under Section 3 for all purposes of this Act, but shall authorize distribution and use only within such State.*

(2) A registration issued by a State under this subsection shall not be effective for more than ninety days if disapproved by the Administrator within that period. Prior to disapproval, the Administrator shall, *except as provided in paragraph (3) of this subsection, advise the State of the Administrator's intention to disapprove and the reasons therefor, and provide the State time to respond. The Administrator shall not prohibit or disapprove a registration issued by a State under this subsection (A) on the basis of lack of essentiality of a pesticide or (B) except as provided in paragraph (3) of this subsection, if its composition and use patterns are similar to those of a federally registered pesticide.*

(3) In no instance may a State issue a registration for a food or feed use unless there exists a tolerance or exemption under the Federal **Food, Drug, and Cosmetic Act that permits the residues of the pesticides on the food or feed. If the Administrator determines that a registration issued by a State is inconsistent with the Federal Food, Drug, and Cosmetic Act, or the use of a pesticide under a registration issued by a State constitutes an imminent hazard, the Administrator may immediately disapprove the registration.**

(4) *If the Administrator finds, in accordance with standards set forth in regulations issued under section 25 of this act, that a State is not capable of exercising adequate controls to assure that State registration under this section will be in accord with the purposes of this Act or has failed to exercise adequate controls, the Administrator may suspend the authority of the State to register pesticides until such time as the Administrator is satisfied that the State can and will exercise adequate controls. Prior to any such suspension, the Administrator shall advise the State of the Administrator's intention to suspend and the reasons therefor and provide the State time to respond."*

The EPA also maintains regional offices. The USA is divided into ten regions. These regional offices are responsible to the local areas that serve to regulate emissions. It is through these regions that National Pollutant Discharge Elimination System (NPDES) permits are obtained, maintained and enforced. The requirement to discharge only as allowed by the NPDES permit is standard language on all pesticide labels.

—*When do I come in contact with the FIFRA?* When you use any substance or mixture of substances and intend to prevent, destroy, repel or mitigate any pest you must use a FIFRA registered pesticide. The one most important thing that brings products into FIFRA jurisdiction is claims. Label claims are the determining factor as to whether FIFRA registration is necessary. Products that claim simply to deodorize are not subject to FIFRA even though the likely mechanism of deodorization is killing germs. It is not until the label states that the product will kill germs do FIFRA requirements come into play.

—*When do I come in contact with the Bureau of Alcohol, Tobacco and Firearms (BATF)?* From a chemical perspective, contact with this agency is only necessary when you market a substance that will become a component of an alcoholic beverage. In the case of antimicrobial agents that may be used in such beverages, the first jurisdictional area is the FDA. Once approved by the FDA, the BATF will require an application to amend their regulations at 27 Code of Federal Regulations, but do not require much more than proof of FDA approval for such use. It is possible that the same antimicrobial used in the alcoholic beverage would also have a use that is not a direct food additive. This would still require EPA approval for those uses; however the BATF and EPA jurisdiction do not intersect.

—*What is authorized by the Food and Drug Administration (FDA)?* The FDA's jurisdiction is only over those materials that contact food, are drugs, or are medical devices. Sometimes the distinction between what is in the EPA's jurisdiction and what is in the FDA's is not clear. From FIFRA, the EPA's, definition of pest had an important exception: *except viruses, bacteria, or other micro-organisms on or in living man or other living animals*. This exception prevents the EPA from having jurisdiction over any product that is designed to mitigate, repel or destroy such pests whether they be microbes or not. The FDA regulates such materials. Therefore, the EPA regulates an insect repellent, which is applied to the skin and designed to repel insects not living in or on man. An antimicrobial soap, however, designed to mitigate/destroy a pest that is on man is FDA-controlled as a drug.

In many cases both the EPA and the FDA have jurisdiction. A preservative intended to be used in the paper-making process must undergo two reviews. First, the product must be approved by the FDA as an indirect food additive and listed in 21CFR, or provide some equivalent FDA authorization. This is due to its potential to migrate to food that may be wrapped in the paper. Secondly, the EPA must review it as a pesticide.

To further confuse the issue, cosmetics, which are by law under the purview of the FDA, are required to undergo no mandatory review. As such ingredients in these materials are not formally regulated by either. Materials used to preserve (mitigate microbes) in cosmetics need not be registered by the EPA or the FDA. Many active ingredients that are used to preserve industrial materials, such as coating, must undergo a rigorous registration process. These same ingredients can be used in cosmetics at the discretion of the cosmetic manufacturer; however, he is fully responsible for any adverse effects upon the customer's health caused by the use of the cosmetic. Additionally there is a voluntary disclosure mechanism in the FDA rules to which many cosmetic ingredient manufacturers adhere.

—*Are there any exemptions for over-the-counter drugs?* The FDA regulates drugs whether prescription or over-the-counter (OTC). Prescription drugs must go through a rigorous FDA approval process, regardless of their function and are specifically exempted by the definition of "Pesticide". A pesticide is again:

"... any substance or mixture of substances intended: (a) for preventing, destroying, repelling or mitigating any pest ..."

The product that mitigates a pest "in or on man or animals" is not within the FIFRA jurisdiction. So if the drug is over-the-counter and it is designed to mitigate a pest that is living in or on man, the jurisdiction remains with the FDA. If however, the product is applied to man, but to mitigate a pest that is external to man, such as an insect repellent, the jurisdiction is EPA's. Whether or not the product is exempted from the requirement of FIFRA depends entirely on the claims that the product makes.

—*When is a Drug Master File (DMF) mandatory for an antimicrobial?* There are certain cases for which it may be advisable for a manufacturer of an antimicrobial to file a Drug Master File. When a manufacturer of an active has generated large amounts of data, that data is protected by FIFRA for a period of fifteen years. No other registrant can use that data for a registration without properly compensating the owner. [Note that this data is publicly available through the Freedom of Information process but it cannot be used to obtain a registration]. Submissions to FDA are not protected from use by other applicants and can be cited and used by subsequent applicants for FDA approval. As such, that manufacturer of an active ingredient that is also approved for food uses may want to establish a master file at the FDA in which to keep any confidential data that was submitted to the FDA. This way the data owner may authorize the FDA to use it for their customer's subsequent FDA approvals, but protect it from competitors who would wish to use it to obtain their own FDA approval. The master file is basically a means to protect confidentiality of data submitted to the FDA.

—*Who appoints a registration number?* The EPA assigns registration numbers to products that have successfully passed review. The registration numbers have no meaning in and of themselves. Each company is assigned a number and the product registration number becomes the company number followed by a dash and the consecutive number of the submission. In other words a company's fifth registration application would be their company number followed by a dash five.

Prior to approval and after the EPA's receipt of the pesticide application, a "file symbol" would be assigned to the product for the purposes of tracking the product through the registration process. The file symbol for the above example of a fifth registration would be the company number followed by an "L". The EPA uses the word "REGULATION" (ten letters) to correspond to the number that the product will become, once registered. The letter "L" is the fifth letter in REGULATION.

It is possible to determine information from a registration number, if the registration is a supplemental registration. In supplemental registration the registrant is simply a distributor of another manufacturer's product. In this case the registration number will be the registration number of the primary product, followed by the company number of the distributor.

Every registered product must also have an establishment number on the label. This is the number of the establishment where final processing of the product was done. This establishment number is composed of the company number, followed by a dash and a state or country abbreviation and then the consecutive number of that company's site within that state.

—*Is there an overview on registration fees?* The EPA assesses only a maintenance fee, once per year. The fee is due by January 15th of every year. The first registered product is \$1,225; each subsequent product is an additional \$2,450 until thirty products are registered. From the 31st to the 50th there is no charge; from the 51st through 72nd each is again assessed \$2,450 until a cap of \$95,000. Anything over the cap is assessed no charge.

During the reregistration process each active that required review was assessed a significant fee, but that was a one-time assessment to cover additional resources required to complete the process and has not been repeated.

There have been many discussions and proposals over the years to allow the EPA to go to a fee-for-service registration structure. Industry, and in particular the antimicrobial registrants, have been resistant to this without some mandated guarantees associated with the fee, such as hard and fast review periods and more explicit registration criteria.

—*When the active substance is not listed in the EPA's Inventory of the Toxic Substance Control Act (TSCA), is it advisable to have it notified prior or parallel to the registration step?* The Toxic Substance Control Act exempts "pesticides" from the requirements of the Act. This is not to say however, that it is not necessary to list the material on the TSCA inventory. If, for instance, paint is being shipped into the United States and the labeling (including all literature) makes no antimicrobial claims, the paint does not need to be registered under FIFRA. All ingredients of the paint, however, must be listed by TSCA, including the preservative. This is true whether or not the preservative is registered. If the preservative is an unregistered pesticide, it may be used to preserve a paint outside the USA and the paint may be brought into the USA, but all ingredients must still be listed on TSCA.

If the paint is preserved with a registered pesticide and still makes no antimicrobial claims for the paint, the registered pesticide has not been used as a pesticide in the USA and is therefore not technically a pesticide. As such, it must be treated as any other ingredient in the paint and be present on the TSCA Inventory before it can be imported. On the other hand, if the paint does make an antimicrobial claim, such as an antifoulant, the entire product must be registered by FIFRA and it is entirely exempted from any TSCA Inventory requirement.

These situations pose the problem of what constitutes a pesticide claim and what does not. If, for example, the claim is that the product contains an antimicrobial for the protection of the product itself, no registration of the product is necessary. If, on the other hand, the antimicrobial is present to protect or disinfect beyond

the container itself such as a surface to be cleaned or an article to which it is incorporated, registration will be required.

—*Can I export my microbiocide, registered or not, to other countries?* FIFRA allows that unregistered pesticides may be manufactured in the USA for export only. The EPA defines these requirements in a policy statement published in 1993 [22]. These requirements include:

- Labeling the material must in general meet the requirement of FIFRA for both hazard identification and understandability.
- Certain parts of the hazards must be in an acceptable business language of the importing country.
- A statement indicating that the product is not, is no longer registered for a specific use, etc. must be on the label.
- An acknowledgment statement, signed by the foreign purchaser must be received and submitted to the EPA prior to the export.

Additionally, the EPA requires import tolerances to be established for residues of unregistered pesticides present on imported commodities. This prevents what some have termed the “circle of poison” or allowing the export of unregistered pesticides only to have them return as residual on imported agricultural products.

—*Do other countries have registration requirements also?* While a product that is unregistered in the USA may be sent to other countries, care should be taken to ensure that the requirements of the importing country are adequately met. Registration requirements in countries other than the USA range from no requirements, through extremely intense.

Most recently, in Europe a patchwork of varying requirements, which included EINECS listing for antimicrobials, are being brought together for antimicrobial pesticides in the Biocidal Products Directive (BPD). When fully implemented it will be the highest level requirement to date.

—*Is there a harmonization of the requirements between the countries of individual registrations, possibly a mutual recognition of authorization?* The Food and Agriculture Organization (FAO) of the United Nations began harmonization efforts in the late seventies. The result was a “Code of Conduct” and a series of guidelines regarding the distribution of pesticides throughout the world. While supported by countries with established regulatory programs, they were not yet willing to modify established regulatory procedure to incorporate these recommendations. The result was that developing countries adopted these procedures and recommendations, while the industrialized countries provided data and expertise to the committee.

More recently, however, with public pressure to bring previously registered pesticides up to today’s testing standards and to provide faster registration for “reduced risk” pesticides, developed countries are forced to consider harmonization projects. The USA and Canada have developed a joint review process for reduced risk pesticides that uses a shared review process. This process has initially worked well in agricultural application but has yet to be tested for an antimicrobial active ingredient. In addition a North American label is being tested in this process under the auspices of the North American Free Trade Agreement (NAFTA). This is a label for a product that will be acceptable to the USA, Canada and Mexico.

There are several other efforts worth mentioning that are not specifically pesticide, but do overlap.

- The Prior Informed Consent (PIC) program, while not really a regulatory program is an information sharing effort which requires exporting countries to be aware of the restrictions or regulations of destination countries. Section 17 of FIFRA has a similar provision that requires a foreign purchaser to acknowledge the receipt of an unregistered pesticide. Future FIFRA amendments are likely to be harmonized with the PIC provisions.
- Endocrine chemical programs in both the USA and Europe are attempting to screen, identify and test chemicals capable of causing this effect. Many pesticides are contained on screening recommendation lists and some form of this testing is likely to become a part of pesticide registration requirements.
- Programs to identify Persistent Organic Pollutants (POPs) and Persistent Bio-accumulative and Toxic (PBT) chemicals are being incorporated into international chemical review programs. Agreements are being made to phase out certain pesticides that meet certain criteria.
- The High Production Volume (HPV) challenge is a voluntary program to evaluate the chemical with the highest production in the USA and Europe. Volumes that triggered inclusion on the HPV list were obtained through the TSCA’s Inventory Update, a requirement that manufactures report the production volumes of certain chemicals every four years. Although antimicrobials products are exempt from the requirements of TSCA several HPV listing resulted due to other, nonexempt uses. Critical decisions on how to support these chemicals in the HPV testing process without affecting data compensation had to be made.

—*Are there any other information reporting requirements in FIFRA?*

Section 6(a)(2) of FIFRA simply states the following:

“if at any time after the registration of a pesticide the registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit such information to the Administrator.”

The intent of this law was to provide the EPA with information not specifically required in the registration process that might be relevant to the EPA’s decision to register.

The EPA’s first guidance concerning this law was in 1978 [23]. In 1979 they published a “Statement of Enforcement Policy” [24] granting exemptions for certain information considered to be unnecessary. The EPA published further interpretation in 1985 [25] and said that it would announce the effective date. That date was never announced, so it is not clear if that guidance was ever in effect. Finally, in 1992 the EPA published a proposed rule pertaining to 6(a)(2) [26]. This proposed rule was not finalized; the comment period was reopened in 1996 and was codified in 1998 [27]. This final rule also revoked all other rules and interpretations.

Some important new provisions of the new guidance are as follows:

- The definition of pesticide was expanded to include “contaminant” as stated in the 1996 draft the definition becomes: “...a pesticide product which is or was registered by EPA, and each active ingredient, inert ingredient, impurity, metabolite, contaminant or degradate contained in or derived from, such pesticide product.”
- The definition of “registrant” was expanded to include: “any person who holds, or ever held, a registration for a pesticide product issued under FIFRA section 3 or 24(c).”
- The rule emphasizes that the registrants of pesticides are responsible for the compliance of their agents.
- The definition of “water reference level” or the level which triggers a report if found in surface or ground water, has been modified. The level depends on the levels established in section 304 of the Clean Water Act, the Maximum Contaminant Level (MCL) established by the EPA, the Health Advisory Level (HAL) or the lowest detectable limit depending on the chemical.
- Information required to be submitted includes incidents.
- The rule emphasizes that the attorney-client privilege is not protected from the requirement to report.
- Retrospective incident reports had to be filed from January 1, 1994 if a hospitalization or fatality is involved. Retrospective environmental reports were required if they meet certain criteria.

Again, with this regulation the EPA provided initial problematic guidance, followed by a clarifying proposal. In the final clarification important definitions were expanded from the original FIFRA definitions. This caused registrants to make decisions on retrospective analyses of data to see if reportable information on degradates or metabolites, thought previously to be non-reportable is now subject. Also, the change in definition of registrant required former manufactures to be subject once again to FIFRA. As a proposal this regulation left the situation extremely vague for several years. The rule was finally enacted regulations but did not provide retroactive relief to those who may have misinterpreted the original rules. These types of issues are not specific to FIFRA, but are prevalent throughout all of EPA rulemaking.

4.1.5 Conclusion

Registrants attempting to traverse the FIFRA registration process should first have plenty of time, money and patience. They should realize that even a critical analysis of the current regulation will not bring them to correct conclusions about the requirements. Proposed rules, former action, Pesticide Registration Notices, re-registration activities, environmental activist priorities and the demeanor of the reviewer must all be taken into consideration. Grey areas exist in almost all regulatory text parts, for instance from how to construct a confidential statement of formula that sufficiently addresses both the entering and final ingredients, to what temperature should be used for an anaerobic aquatic metabolism study to make it useful in all venues.

The successful applicant will have frequent discussions and meetings with the EPA. This will be the first test of patience. These discussions and meetings should reach a conclusion and be documented and agreed to by both parties, but the EPA will seldom provide or agree to meeting minutes. Good follow-up, technical capabilities and persuasive skills are required by the applicant. In particular, additional data requirements outside of the federally coded requirements should be firmly established.

If the registration is for a technical grade active ingredient, the registrant should work closely with the end user(s) to identify and quantify exposures. It is often quantitative exposure information that can justify the waiver of expensive studies or mitigate significant contribution to the “risk cup” enough to justify additional approvals. This becomes more apparent as expensive antimicrobial data requirements force lower volume actives out of the market in favor of actives that also have agricultural application and can support extensive data bases. These products however must compete for space in aggregate risk cups.

Continual monitoring of the post submission activities is also critical. Each mailing should be followed by letters of receipt and understanding of the type of review that the EPA is conducting. FQPA review time mandates should be agreed upon verbally with the reviewer assigned to the registration. Submissions are often lost in the bureaucracy between the Document Control Desk, where submissions are received, and the desk of the product manager. Valuable review time can be saved by assuring that the Product Manager actually receives the submission.

While no two registrations are ever the same and no two reviewers ever seem to view the somewhat subjective data and labeling requirements the same way, incremental progress is being made. The creation of the Antimicrobial Division and the recognition that there is this other class of products that do not have the same exposure and risk characteristics as classical pesticides that may be broadcast across acres of land to serve their function and sprayed in the consumer's home to mitigate insects, is a large first step. Even though the new antimicrobial regulation and data requirements are not developing as quickly as might be hoped, the antimicrobial industry is able to focus on the Antimicrobial Division progress in this area unencumbered by the requirements for conventional agricultural pesticides.

References

1. Reorganization Number 3 of 1970, §2(a)(1), 1970, 91st U.S. Congress, second session.
2. Public Law No. 92-516, 86 Stat. 973, 1972.
3. Public Law No. 104-170, 110 Stat. 1489, 1996.
4. 51 FR 33992, September 24, 1986.
5. 36 Stat. 331 Ch. 191, 1910.
6. DDT was a successful insect control agent that was later found to have adverse effects on the thickness of birds' eggshells.
7. Public Law No. 100-532, 102 Stat. 2654, 1988.
8. 40 CFR 160, Good Laboratory Practice Standards.
9. 52 FR 13305, 58 FR 48414 and 52 FR 49400.
10. EPA Label Review Manual, December 27, 1996.
11. PR Notice 98-1 - Self-Certification of Product Chemistry Data with Attachments.
12. Data Call-In Notice For Subchronic and Chronic Toxicological Data For Antimicrobial Pesticide Active Ingredients, Office of Pesticide Programs, March 4, 1987.
13. Popen Dorf, W., Selim, M. and Kross, B. (1992) Chemical Manufacturer's Association Antimicrobial study: Lab Project Number: Q626. Unpublished Study Prepared by the University of Iowa. 316 p.
14. 40 CFR 125 - CRITERIA AND STANDARDS FOR THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES).
15. PESTICIDE REGULATION (PR) NOTICE 95-1 Effluent Discharge Labeling Statements.
16. General Information On Applying For Registration Of Pesticides In The United States, Second Edition; EPA/737/b-92-001; Office of Pesticides and Toxic Substances (H-70505C).
17. 40 CFR Subpart A.
18. Public Law No. 94-46g, 90 Stat. 2003.
19. Public Law No. 104-170, 110 Stat. 1501, 1996.
20. Pesticide Registration (PR) Notice 97-3, September 4, 1997.
21. 67 FR 8244, February 22, 2002.
22. 58 FR 9062.
23. 43 FR 37611.
24. 44 FR 40716.
25. 50 FR 38115.
26. 57 FR 44290.
27. 40 CFR 159.

Glossary

ACC	American Chemistry Council (formerly Chemical Manufacturer Association (CMA))
BATF	Bureau of Alcohol, Tobacco and Firearms
BPD	Biocidal Products Directive
CFR	Code of Federal Regulations
CSF	Confidential Statement of Formula
CWA	Clean Water Act
DCI	Antimicrobial Data Call-In
DMF	Drug Master File
EP	End-use Product
EPA	Environmental Protection Agency
EUP	Experimental Use Permit
FAO	Food and Agriculture Organization (of the United Nations)
FCN	Food Contact Notification
FDA	Food and Drug Administration

FFDCA	Federal Food, Drug and Cosmetics Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
GLP	Good Laboratory Practice
HAL	Health Advisory Level
HPV	High Production Volume
IMO	International Maritime Organization
MCL	Maximum Contaminant Level
MOE	Margin of Exposure
MUP	Manufacturing Use Product
NAFTA	North American Free Trade Agreement
NPDES	National Pollutant Discharge Elimination System
OTC	Over-the-Counter (Drugs)
PBT	Persistent, Bioaccumulative and Toxic
PIC	Prior Informed Consent
POP	Persistent Organic Pollutant
PR-Notice	Pesticide Registration Notice
Regulation	Keyword for numbering 1-2-3-4-5-6-7-8-9-0
SLN	Special Local Needs
TGAI	Technical Grade Active Ingredient
TSCA	Toxic Substances Control Act
USDA	United States Department of Agriculture

4.2 The European biocidal products directive

BIRGIT SCHMIDT-SONNENSCHIN

4.2.1 Introduction

During the Council discussion on Directive 91/414/EEC concerning the placing of plant protection products on the market [1] the European Council expressed concern at the lack of harmonised Community provisions for biocides. The Commission reviewed the situation in the Member States, establishing that there are differences not only regarding trade in biocidal products but also regarding trade in products treated with them, thereby affecting the functioning of the internal market.

The Commission proposed therefore the development of a framework of rules related to the placing on the market for use of biocidal products.

A directive on the placing of biocidal products on the market was considered the most appropriate way to establish such a framework.

Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market was adopted on February 16, 1998 [2], published in the Official Journal on April 24, 1998 and enacted on May 14, 1998.

Through this Directive biocidal products and the active substances they contain are regulated in Europe for the first time in a harmonised evaluation and authorisation process.

The main aims of the Biocidal Products Directive (BPD) are:

- the harmonisation of the common market for biocides,
- and
- a high level of protection for humans, animals and the environment.

But to what extent the aim of a European harmonisation can be achieved is dependent on how the Biocidal Products Directive will be implemented in the different Member States into their national laws and whether the competent authorities will act in a harmonised manner.

The Biocidal Products Directive is a comprehensive European regulation on biocides with antimicrobial efficacy but also on pest control products such as insecticides, rodenticides, molluscicides, avicides, repellents and others.

The Biocidal Products Directive strongly regulates biocidal products before they are placed on the market in a similar way to plant protection products. The basis of the decision by the authorities is the evaluation of all risks for man, animals and the environment arising from the use of a biocidal product and the assessment of whether these risks are acceptable or not.

4.2.2 Content of the biocidal products directive

Definition of a biocidal product

For the purposes of the Biocidal Products Directive biocidal products are defined as

“Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means”.

In this connection the purpose advertised is of particular importance, that means how the product attributes are offered publicly. Products which are placed on the market with an appropriate indication like disinfectant, bactericidal, antimicrobicidal or wood-protecting are normally considered biocidal products for the purpose of this Directive. But besides the public offer the expectation of the purchaser also plays an important role.

Annex V of the Directive includes an exhaustive list of 23 product types covered by the Directive with a description of examples (see Table 4).

Scope

The Biocidal Products Directive applies to these 23 biocidal product types but excludes certain biocidal products that are defined or within the scope of instruments for the purposes of other Community directives listed

in Article 1 paragraph 2 a - r of the Directive. The main directives regulating biocidal products which are excluded from the scope of the Biocidal Products Directive are:

- Directives relating to medicinal products, veterinary medicinal products and medical devices,
- Directives concerning food additives, flavourings and additives in feedstuffs,
- Directives relating to materials and articles intended to come into contact with foodstuffs,
- Directives related to hygiene and health rules for the production and placing on the market of foodstuffs,
- Directive relating to cosmetic products,
- Directive concerning the placing of plant protection products on the market.

But it has to be recognised that these existing EC directives have been implemented in the Member States in different ways, with one defining a product as a medicinal product, while others the same product as a biocidal product or a cosmetic product.

Many borderline cases have already been identified and guidance documents have been elaborated for the most important cases to give practical guidance and examples.

Borderline documents and other guidance on scope issues relevant to the Biocidal Products Directive are published on the website of the GD ENV (<http://europa.eu.int/comm/environment/biocides/index.htm>).

- Borderline between biocidal products, proprietary medicinal products and veterinary medicinal products,
- Borderline between biocidal products and plant protection products,
- Borderline between biocidal products and cosmetic products,
- Borderline between biocidal products and milk hygiene legislation,
- Guidance document on treated material/articles and some other scope issues (human hygiene biocidal products, private area and public health area disinfectants),
- Guidance document regarding the in-situ generation of biocidal active substances.
- Guidance document regarding mode of action and other issues.

But these documents on borderline cases do not oblige the Member States to adopt the same attitude and they are not legally binding since only the Court of Justice can give an authoritative interpretation of existing Community law.

Authorisation for placing on the market of biocidal products

The placing on the market of products for use as biocidal products is subject to authorisation by the Member States.

For an authorisation of a biocidal product the applicant has to submit a dossier (or a letter of access)

- for the biocidal product satisfying the requirements set out in Annex IIB and the relevant parts of Annex IIIB
- for each active substance in the biocidal product satisfying the requirements set out in Annex IIA and the relevant parts of Annex IIIA

to the competent authority of the Member State where the biocidal product is to be placed on the market for the first time. The applicant is required to have a permanent office within the Community.

The dossiers shall include a detailed and full description of the required studies conducted and of the methods used.

But there is no need to supply information

- which is not necessary owing to the nature of the biocidal product or of its proposed uses,
- which is not scientifically necessary or not technically possible to obtain.

The competent authority can ask the applicant to submit further information, if it deems it necessary to evaluate the risks of the biocidal product.

All studies must be conducted according to the methods described in Annex V to Directive 67/548/EEC [3] under the provisions of Council Directive 87/18/EEC [4] relating to the application of the principles of good laboratory practice. Deviations from these requirements must be justified and will be decided on a case-by-case basis.

By way of derogation from this, Member States shall allow the placing on the market and use of

- low-risk biocidal products provided they have been subject to registration,
- basic substances for biocidal purposes once they have been included in Annex IB.

The registration procedure is only possible for low-risk biocidal products. Low-risk biocidal products contain as active substances only one or more of those listed in Annex IA and do not contain any substance(s) of concern. Under the conditions of use, such biocidal products may pose only low risk to humans, animals and the environment.

See also Chapter 4.3. Protection of health – Microbicides in the environment.

The registration dossier for a low-risk biocidal product need not meet the requirements set out in Annexes IIB and IIIB for the products and IIA and IIIA for the active substance(s). For registration the dossier comprises only data on

- the applicant,
- the identity of the biocidal product,
- the intended uses,
- efficacy data,
- analytical methods,
- classification, packaging and labelling,
- safety data sheet.

The competent authority shall take a decision on registration within a period of 60 days.

Basic substances are substances which are listed in Annex IB whose major uses are non-biocidal but which have some minor uses as biocides either directly or in products consisting of a basic substance and a simple diluent which itself is not a substance of concern. The basic substance is not directly marketed for a biocidal use, but can be used directly for biocidal purposes without further authorisation or registration of the product.

For inclusion under Annex IB the same requirements laid down in articles 10 and 11 of the Biocidal Products Directive have to be fulfilled as for active substances intended for inclusion in Annex I or IA.

Member States will authorise a biocidal product only if

- the active substance(s) included are listed in Annex I of IA of the Directive,

and

- according to the common principles for the evaluation of dossiers as laid down in Annex VI the biocidal product
 - has no unacceptable effects on human or animal health,
 - has no unacceptable effects on the environment,
 - is sufficiently effective,
 - has no unacceptable effects on the target organisms, such as unacceptable resistance or unnecessary suffering and pain for vertebrates.

If a biocidal product is classified as toxic, very toxic, carcinogenic, mutagenic or toxic for reproduction (category 1 or 2) it shall not be authorised for marketing to, or use by the general public.

Authorisation of a biocidal product will be granted for a maximum period of 10 years; but it may be reviewed at any time, if there are new knowledge or information on the effects for humans or the environment.

Frame formulations

Frame formulations are defined as

“Specifications for a group of biocidal products having the same use and user type.”

Such a group of products must contain the same active substance(s) of the same specification(s), and their compositions may present only variations from a previously authorised biocidal product which do not affect the level of risk associated with them and their efficacy.

In this context, a variation can be

- a reduction in the percentage of the active substance and/or
- an alteration in percentage composition of one or more non-active substances and/or
- the replacement of one or more pigments, dyes or polymers by others presenting the same or a lower risk, but the efficacy of the biocidal product may not be decreased.

Mutual recognition

If a biocidal product has already been authorised or registered in one Member State it is necessary to authorise/register it again in the Member State(s) where the biocidal product is also to be placed on the market.

The applicant must submit

- a summary of the dossier for the biocidal product,
- a summary of the dossier for the active substance(s) according to Annex IIB, Section X,
- a certified copy of the first authorisation granted.

The second or further Member State(s) shall authorise or register the biocidal product within 120 days, or 60 days respectively.

If the second or further Member State establishes that the target species is not present in harmful quantities, or unacceptable tolerance or resistance of the target organism is demonstrated, or the relevant circumstances of use, such as climate or breeding period of the target species, differ significantly from those of the first Member State's authorisation, the Member State may request the adjustment of the labelling requirements according to

- the directions for use and the dose rate,
- the particulars of likely direct or indirect adverse side effects and any directions on first aid,
- the directions for safe disposal of the biocidal product and its packaging,
- the particulars concerning waiting period, decontamination means and measures during use, storage and transport,
- information on any specific danger to the environment particularly concerning protection of non-target organisms and avoidance of contamination of water.

The complete refusal or restriction of an authorisation of a biocidal product or a registration of a low-risk biocidal product has to be notified to the Commission, other Member States and the applicant. The Commission has to prepare a draft decision under consideration of the comments by other Member States within 90 days. The applicant shall be asked to submit remarks. A final decision will be adopted by the representatives of all Member States chaired by a representative of the Commission (Standing Committee).

Provisional authorisation

Biocidal products may be authorised under certain circumstances

- temporarily for a period not exceeding 120 days, for a limited and controlled use in case of an unforeseen danger which cannot be contained by other means;
- provisionally for a period not exceeding three years, if the biocidal product contains a new active substance, not yet listed in Annex I or IA, but where the dossier of the active substance has been evaluated as satisfying the requirements, and the biocidal product may be expected to be authorised.

Inclusion of an active substance in Annex I or IA

Only those biocidal products can be authorised or registered which contain exclusively active substances which are listed in Annex I or IA.

For the inclusion of active substances in Annex I or IA the applicant has to submit complete dossiers satisfying the data requirements laid down in

- Annex IIA (common core data set for active substances: chemical substances),
- Annex IIIA (additional data set for active substances: chemical substances), or
- Annex IVA (data set for active substances: fungi, micro-organisms and viruses),

and a dossier for at least one biocidal product containing the active substance.

The common core data set is mandatory for all chemical active substances. Subject to the use of the biocidal product containing the active substance, additional data according to Annex IIIA are necessary.

An active substance will be included in Annex I, IA or IB only if it is confirmed through a risk assessment that the substance under the conditions of use of the biocidal product, the use of the treated material (if appropriate) and the consequences from use and disposal has no unacceptable effects on humans, animals and the environment.

The active substance will be included in the Annexes for an initial period not exceeding 10 years. The inclusion may be renewed on one or more occasions for periods not exceeding 10 years.

At any time the initial inclusion as well as any renewed inclusion may be reviewed, if there is new knowledge or information on the effects of the active substance for humans or the environment influencing the result of the risk assessment.

Active substances (for low-risk biocidal products) cannot be included in Annex IA if they are classified according to Directive 67/548/ECC [3] as

- carcinogenic,
- mutagenic,
- toxic for reproduction,
- sensitising,
- bioaccumulative
- not readily degradable.

The substitution principle

An entry of an active substance in Annex I, IA or IB may be refused or removed, if the evaluation of the active substance shows that under normal use conditions risks to health or the environment still give rise to concern, and if there is another active substance on Annex I for the same product type available, which presents significantly less risks to health or to the environment.

But it has to be taken into account that the alternative active substance can be used with similar effects on the target organism without economic and practical disadvantages for the user and without an increased risk for health and for the environment.

The assessment shall be carried out under the following conditions:

- the chemical diversity of the active substances should be adequate to minimise occurrence of resistance,
- application to active substances which present a significantly different level of risk,
- application to active substances used in products of the same product-type,
- applications only after allowing the possibility of acquiring experience from use in practice.

The decision to remove an Annex I entry shall be delayed for a period up to a maximum of four years from the date of the decision.

Data protection

The data protection provisions are contained in Article 12 of the Directive. The purpose of data protection provisions is to allow applicants who have supported the inclusion of active substances in Annex or IA of the Directive (alone or as part of a consortia) to recover some of the costs of generating the data. Therefore many Member States only use the information referred to in Article 8 for the benefit of a second or subsequent applicant if this applicant has access to the data or the data protection has expired for the data.

The access to the data has to be proved by a written agreement in the form of a letter of access of the first applicant.

The data protection periods according to Article 12 for active substances and biocidal products are as described in Table 1.

Table 1 Data protection periods.

Type	Time frame	Conditions
New active substances	15 years	from the date of first inclusion in Annex I or IA
Existing active substances:		from May 13, 2000
• information for the purposes of the Directive, unless such information is already protected under existing national rules related to biocidal products	10 years	depending on remaining period of data protection provided for under national rules
• information submitted for the first time in support of the first inclusion in Annex I or IA either for the active substance or an additional product type for that active substance	X years	from the date of entry onto Annex I or IA
• information submitted for the first time in support of the first inclusion in Annex I or IA either for the active substance or an additional product type for that active substance	10 years	
• further information for the first submission:	5 years	from the date of decision unless the five-year period expires before the period of 15 or 10 years (mentioned above), in which case the period shall be extended so as to expire on the same date
– variation of requirements		
– maintenance of the entry of Annex I or IA		
Biocidal products		
• containing a new active substance	10 years	from the date of first authorisation in any Member State
• containing an existing active substance	10 years	same conditions as for existing active substances taking into account if there are already national protection rules applicable
• further information submitted for the first time	5 years	same conditions as for existing active substances

Research and development

For the purposes of research and development involving the placing on the market of an unauthorised biocidal product or a new active substance intended exclusively for the use in a biocidal product, derogations from the authorisation rules are possible:

- In the case of scientific research and development written records have to be maintained detailing the
 - identity of the biocidal product or active substance,
 - labelling data,

- quantities supplied,
- names and addresses of all persons receiving the biocidal product or active substance,
- dossier on all available data of possible effects on human or animal health or impact on the environment.

This information shall only be made available to the competent authority on request.

- In the case of process-oriented research and development the same information as described above has to be notified to the competent authority where and before placing on the market occurs and to the competent authority of the Member State where the experiment or test is to be conducted.
- In the case that the experiment or test may result in release into the environment of the unauthorised biocidal product or active substance the competent authority has to issue an authorisation which limits the quantities to be used and the areas to be treated.

Confidentiality

For the protection of commercially sensitive information the applicant may indicate that which he wishes to be kept confidential from all persons other than the competent authorities and the Commission.

Full justification is required of why disclosure of the information might harm the applicant industrially or commercially. Information accepted as confidential by the receiving competent authority shall be treated as confidential by the other competent authorities, Member States and the Commission.

In any case the following information is not considered as confidential:

- name and address of the applicant;
- names and addresses of the manufacturers of the biocidal product and/or active substance(s);
- names and content of the active substance(s) in the biocidal product and the name of the biocidal product;
- names of substances classified as dangerous and which contribute to the classification of the product;
- physical and chemical data of the active substance(s) and the biocidal product;
- any ways of rendering the active substance or biocidal product harmless;
- efficacy data concerning the active substance(s) or biocidal product;
- effects of the active substance(s) or biocidal product on humans, animals and the environment and, where applicable, its ability to promote resistance;
- methods and precautions to reduce dangers from handling, storage, transport and use as well as from fire or other hazards;
- safety data sheets;
- methods of analysis;
- methods of disposal of the product and of its packaging;
- first aid and medical advice.

Classification, packaging and labelling of biocidal products

Biocidal products have to be classified, packaged and labelled according to the provisions of Directive 88/379/EEC [5]. Additionally the label of each biocidal product must show:

- identity of every active substance and its concentration;
- authorisation number allocated by the competent authority;
- type of preparation (e.g. granules, powder, liquid concentrate);
- the use for which the product is authorised (e.g. wood preservation, pest control, disinfection, etc.);
- directions for use and the dose rate for each authorised use;
- likely direct or indirect adverse side effects and any directions for first aid;
- if accompanied by a leaflet, the sentence “Read attached instructions before use”;
- directions for safe disposal of the biocidal product;
- the formulation batch number or designation and the expiry data relevant to normal conditions of storage;
- period of time needed for the biocidal effect, the interval needed between applications, or the next access to the area where the biocidal product has been used, including means and measures of decontamination;

and where applicable:

- categories of use (e.g. industrial, professional);
- information on specific danger to the environment particularly concerning protection of non-target organisms;
- for microbiological biocidal products, labelling requirements according to Council Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work [6].

Labels of a biocidal product shall not be misleading and shall not mention indications like “low-risk biocidal product”, “non-toxic”, “harmless” or similar indications.

Advertising

Every advertisement for a biocidal product shall be accompanied by the sentences “Use biocides safely. Always read the label and product information before use.” No advertising of a biocidal product shall mention “low-risk biocidal product”, “non-toxic”, “harmless” or any similar indications.

Poison control

Information on the chemical composition of every biocidal product has to be submitted to the national poison control centres (appointed by the Member States) for the purpose of suspected poisoning arising through biocidal products. This information may only be used to meet medical demand, in particular in emergencies, and may not be used for other purposes. Member States have to ensure that the information is kept confidential.

Contrary to the provisions of the Dangerous Preparation Directive information on every biocidal product, irrespective of whether it is classified as dangerous or not and whether it is intended for professional or consumer use, has to be submitted to the poison control centres.

The Annexes.

Annexes I, IA, IB.

Annexes I, IA and IB are empty at the moment.

Annex I will include the list of active substances evaluated at Community level and allowed for the agreed use in biocidal products.

Annex IA will include only those active substances allowed for the agreed use in low-risk biocidal products.

Annex IB will include basic substances which have some minor use as a biocide but whose major use is non-biocidal (e.g. carbon dioxide, ethanol, acetic acid, etc).

Annexes IIA, IIB.

Annex IIA includes the common core data set for chemical active substances.

The dossier requirements are described in Table 2.

Annex IIB includes the common core data set for chemical biocidal products.

The dossier requirements are described in Table 3.

Table 2 Dossier requirements for chemical active substances.

I	Name and address of applicant and active substance manufacturer
II	Identity of the active substance
III	Physical and chemical properties of the active substance
IV	Analytical methods for detection and identification
V	Effectiveness against target organisms and intended uses
VI	Toxicological profile for man and animals including metabolism
VII	Ecotoxicological profile including environmental fate and behaviour
VIII	Measures necessary to protect man, animals and the environment
IX	Classification and labelling
X	Summary and evaluation of sections II to IX

Table 3 Dossier requirements for chemical biocidal products.

I	Name and address of the applicant and the formulator of the biocidal product and the active substance(s)
II	Identity of the biocidal product
III	Physical and chemical properties of the biocidal product
IV	Methods for identification and analysis of the biocidal product
V	Intended uses of the biocidal product and efficacy for these uses
VI	Toxicological data for the biocidal product (additional to that for the active substance)
VII	Ecotoxicological data for the biocidal product (additional to that for the active substance)
VIII	Measures necessary to protect man, animals and the environment
IX	Classification, packaging and labelling
X	Summary and evaluation of Sections II to IX

Annexes IIIA and IIIB.

Annex IIIA includes the additional data set for chemical active substances.

The requirements include:

- further physical and chemical properties;
- analytical methods for detection and identification in/on food or foodstuffs and other products where relevant;
- further toxicological studies (e.g. neurotoxicity);
- further ecotoxicological studies (e.g. acute toxicity on non-aquatic, non-target organisms, effects on birds, prolonged toxicity to fish);
- identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater [7];
- further human health-related studies;
- further studies on fate and behaviour in the environment.

Annex IIIB includes the additional data set for chemical biocidal products.

The requirements include:

- further human health studies;
- further studies on fate and behaviour in the environment;
- further ecotoxicological studies.

Annexes IVA and IVB.

Annex IVA includes the data requirements for active organisms such as fungi, micro-organisms and viruses and Annex IVB for biocidal products containing such active organisms.

Annex V.

Annex V includes an exhaustive list of 23 product types with an indicative set of descriptions within each type. But these product types exclude products where they are covered by other Directives for the purposes of these Directives. The 23 product types are listed in Table 4.

Table 4 Biocidal product-types as referred to in article 2(1)(a) of the Biocidal Products Directive.

Main group 1:	Disinfectants and general biocidal products
Product-type 1:	Human hygiene biocidal products Used to reduce concentration of biological agents in the body.
Product-type 2:	Private area and public health area disinfectants and other biocidal Products Used for the disinfection of air; surfaces, materials, equipment and furniture and not used for direct food or feed contact in private, public or industrial areas, including hospitals, as well as products used as algicides. Usage areas include swimming pools, aquariums, bathing and other waters; air-conditioning units; walls and floors in health and other institutions; chemicals toilets, waste water, hospital waste, soil and other substrates (in playgrounds).
Product-type 3:	Veterinary hygiene biocidal products Used in areas in which animals are housed, kept or transported.
Product-type 4:	Food and feed area disinfectants Used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food, feed or drink (including drinking water) for humans and animals.
Product-type 5:	Drinking water disinfectants Used for the disinfection of drinking water (for both, humans and animals).
Main Group 2:	Preservatives
Product-type 6:	In-can preservatives Used for the preservation of manufactured products, other than foodstuffs or feedingstuffs, in containers, by the control of microbial deterioration to ensure the shelf life.
Product-type 7:	Film preservatives Used for the preservation of films or coatings in order to protect the initial properties of the surface of materials or objects such as paints, plastics, sealants, wall adhesives, binders, papers, art works etc.
Product-type 8:	Wood preservatives Used for the preservation of wood, from and including saw-mill stage, or wood products by the control of wood-destroying or wood disfiguring organisms (including preventative and curative products).
Product-type 9:	Fibre, leather, rubber and polymerised materials preservatives Used for the preservation of fibrous or polymerised materials, such as leather, rubber, paper or textile products.
Product-type 10:	Masonry preservatives Used for the preservation and remedial treatment of masonry or other construction materials other than wood by the control of microbiological algal attack.
Product-type 11:	Preservatives for liquid-cooling and processing systems Used for the preservation of water and other liquids used in cooling processing systems by the control of harmful organisms such as microbes, algae and mussels (not drinking water preservation products).

Table 4 (Continued)

Product-type 12:	Slimicides Used for the prevention or control of slime growth on materials, equipment and structures, used in industrial processes, e.g. on wood and paper pulp, and porous sand strata in oil extraction.
Product-type 13:	Metalworking-fluid preservatives Used for the preservation of metalworking fluids by the control of microbial deterioration.
Main Group 3:	Pest control
Product-type 14:	Rodenticides Used for the control of mice, rats or other rodents.
Product-type 15:	Avicides Used for the control of birds.
Product-type 16:	Molluscicides Used for the control of molluscs, e.g. snails, which may clog pipes.
Product-type 17:	Piscicides Used for the control of fish. Excludes products for the treatment of fish diseases.
Product-type 18:	Insecticides, acaricides and to control other arthropods, Used for the control of arthropods (e.g. insects, arachnids and crustaceans).
Product-type 19:	Repellents or attractants. Used to control harmful organisms (invertebrates such as fleas, vertebrates such as birds), by repelling or attracting, including those that are used for human or veterinary hygiene either directly or indirectly.
Main Group 4:	Other biocidal products
Product-type 20:	Preservatives for food or feedstocks Used for the preservation of food or feedstuffs by the control of harmful organisms.
Product-type 21:	Antifouling products Used to control growth and settlement of fouling organisms (microbes and higher forms of plant and animal species) on vessels, aquaculture equipment or other structures used in water.
Product-type 22:	Embalming and taxidermist's fluids Used for the disinfection and preservation of human or animals corpses, or parts thereof.
Product-type 23:	Control of other vertebrates Used to control vermin.

Annex VI.

Annex VI describes the “Common Principles for the Evaluation of Dossiers for Biocidal Products”.

This annex lays down the principles for the risk assessment and the evaluation made and the decisions taken by a Member State concerning the authorisation of a biocidal product.

The risk assessment entails the hazard identification and, as appropriate, dose (concentration) response (effect) assessment, exposure assessment and risk characterisation.

This evaluation includes

- effects on humans and animals;
- effects on the environment;
- unacceptable effects;
- efficacy.

An overview of the structure of the Annexes of the Biocidal Products Directive is given in Figure 1.

Summary

According to the Biocidal Products Directive only biocidal products that have been evaluated and authorised can be placed on the common market of the EU.

The decision on authorisation of a biocidal product shall be risk-based.

That means, the health and environmental effects of the active substance and the biocidal product are assessed and compared to the measured or estimated exposure data. The assessment of the efficacy of the biocidal product and the judgement of the need of the intended uses is also taken into consideration.

The authorisation of a biocidal product takes place in two steps:

- active substances are evaluated at the EU level,
- biocidal products are authorised at a national level.

An acceptable active substance will be included into one of the Annexes I, IA or IB of the Directive. The listing is always connected to a specified biocidal product-type (according to Annex V) and is therefore associated with specific conditions of use.

The main prerequisite for the authorisation of a biocidal product will be the inclusion of the active substance to Annex I or IA and, that the biocidal product has no unacceptable effects on human or animal health or on the environment and that it is sufficiently effective.

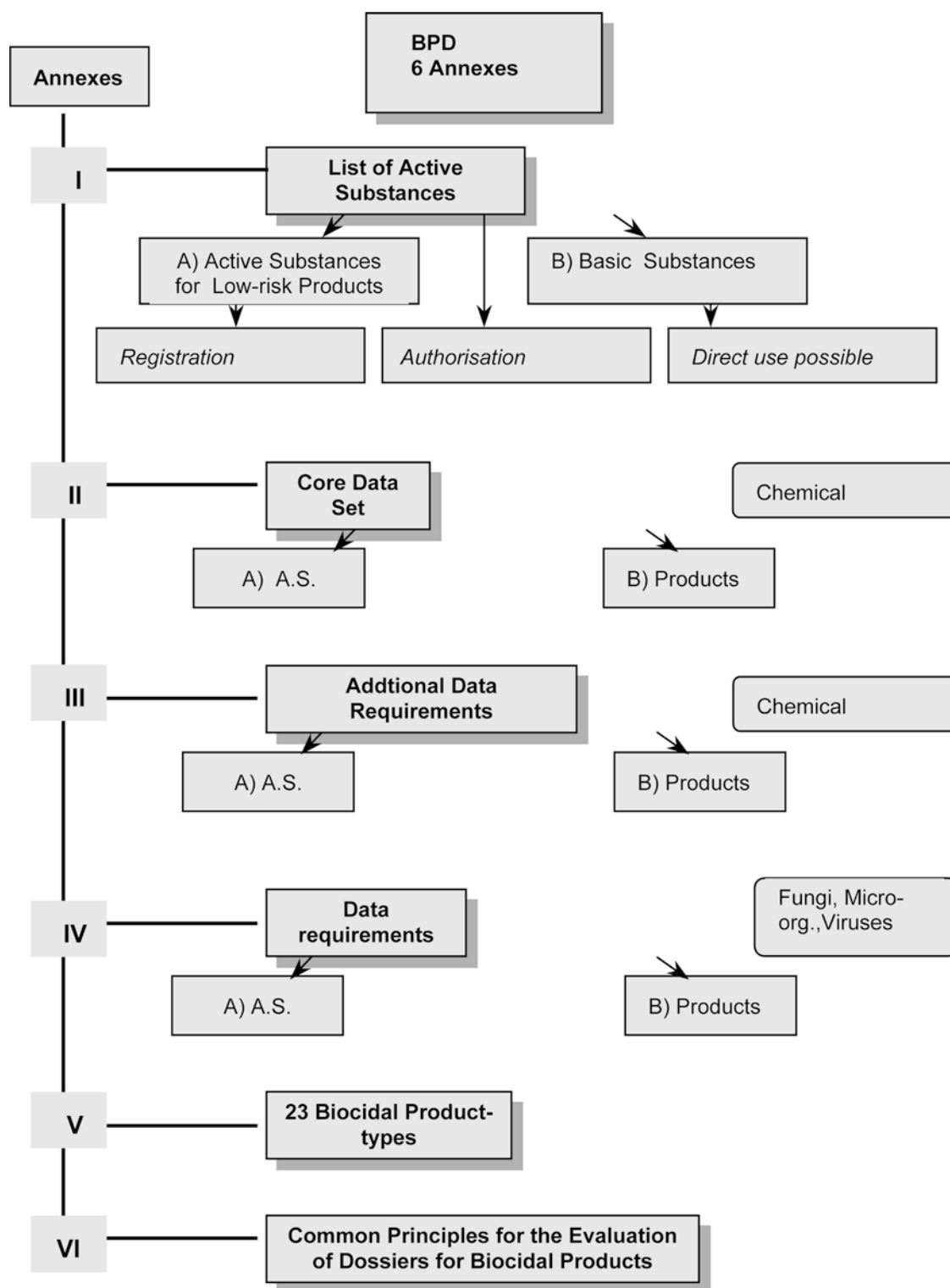


Figure 1 Structure of the BPD Annexes.

Additionally the Biocidal Products Directive also contains provisions on temporary authorisation of products, data protection, research and development, confidentiality, classification, packaging and labelling, advertising and poison control.

4.2.3 Transitional measures and the review programme

Before an authorisation of a biocidal product on national level will take place, the existing active substances will be subject of a systematic examination.

Existing substances are defined as substances that were already on the European market as active substances in biocidal products before May 14, 2000.

Member States may continue to apply their current national rules for the placing on the market of existing active substances and biocidal products containing them in their territory, for a period of 10 years from May 14, 2000.

During the transitional period these active substances will be reviewed and evaluated for possible inclusion in the Annexes I, IA and IB. The details of this 10-year programme of work for the systematic examination are described in two Review Regulations of the European Commission.

The first Review Regulation (Commission Regulation (EC) No 1896/2000 of 7 September 2000 on the first phase of the programme referred to in Article 16 (2) of Directive 98/8/EC of the European Parliament and of the Council on biocidal products [8]) describes the procedures for identification and notification of existing active substances, which had to be submitted before March 28, 2002. With a notification applicants support the evaluation of active substances for listing in the Annexes. Those active substances, for which the notification was accepted, may stay on the market for biocidal purposes until the decision on inclusion or non-inclusion in the Annexes has been taken.

Active substances only identified shall be allowed to stay on the market only for a period of three years from the date the second Review Regulation will come into force.

For active substances which are neither identified nor notified no further phase-out period will be allowed. They will be considered as new substances in the future.

The first Review Regulation has determined that active substances for wood preservatives and rodenticides will be evaluated first in the review process. The complete dossiers for these active substances had to be submitted to the designated competent authorities until March 28, 2004.

The second Review Regulation has come into force in December 2003. (Commission Regulation (EC) No 2023/2003 of 4 November 2003 on the second phase of the 10-year work programme referred to in Article 16 (2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, and amending Regulation (EC) No 1896/2000) [9].

The regulation contains lists of all active biocidal substances identified or notified.

- Annex I contains the exhaustive list of existing active substances which have been identified or notified.
- Annex II contains an exhaustive list of existing active substances for which
 - at least one notification has been accepted by the Commission,
 - a Member State has indicated an interest.

The list specifies the product-type(s) for which a notification has been accepted or a Member State has expressed an interest.

- Annex III contains the list of existing substances that have been identified but in respect of which no notification has been accepted or no Member State has indicated an interest.

Existing active substances that have been identified only (Annex III) will not be evaluated within the review programme and will not be included in Annex I, IA or IB of the Biocidal Products Directive. The same applies to any existing active substance/product-type combination of which a notification has not been accepted. The effective date from which Member States shall cancel existing authorisations and registrations for biocidal products containing the substances listed in Annex III and from which such biocidal products may not be placed on the market is September 1, 2006.

For active substances which have been neither identified nor notified no further phase-out period is allowed for them or for biocidal products containing those substances. From the date of entry into force of the second Review Regulation those substances shall be considered as if the substance was not placed on the market for biocidal purposes before May 14, 2000. That means those substances are considered as new biocidal active substances.

Accepted notified active substances and biocidal products containing them may be placed on the market until the decision on inclusion or non-inclusion in Annex I or IA of those substances has been made. After inclusion of active substances in Annex I or IA biocidal products containing those active substance(s) have to be authorised. If an active substance is not included, the biocidal products containing the active substance have to be withdrawn from the market after a phase-out period defined by the Commission.

Annex V specifies the Rapporteur Member States responsible to review the accepted notified substances. The notified substances in respect to wood preservatives and rodenticides (product-types 8 and 14) and to molluscicides, insecticides, repellants and attractants and antifouling products (product-types 16, 18, 19 and 21) are designated to Rapporteur Member States who are responsible of the evaluation of the dossiers.

The deadline for submission of the dossiers for product-types 8 and 14 was March 28, 2004.

Complete dossiers for product-types 16, 18, 19 and 21 must be received by the competent authority of the Rapporteur Member State no earlier than November 1, 2005 and no later than 30 April 2006.

The deadline for submission of complete dossiers for human hygiene biocidal products, private area and public health area disinfectants and other biocidal products, biocidal products for veterinary hygiene and for

food and feed area disinfectants, drinking water disinfectants, in-can preservatives and metalworking-fluid preservatives (product-types 1, 2, 3, 4, 5, 6 and 13) is between February 1, 2007 and July 31, 2007.

For film preservatives, preservatives for fibre, leather, rubber and polymerised materials, masonry preservatives, preservatives for liquid cooling and processing systems, slimicides, avicides, piscicides, preservatives for food or feedstocks, embalming and taxidermist fluids and biocidal products for control of other vertebrates (product-types 7, 9, 10, 11, 12, 15, 17, 20, 22 and 23) the complete dossiers have to be submitted to the competent authority between May 1, 2008 and October 31, 2008.

Annex IV contains the requirements to prepare a complete dossier and the summary dossier. The dossier must include

- the original test and study reports
- the summary dossier including the list of references used, the risk assessment, the overall summary and assessment, a completeness check by the applicant.

The formats described in the Technical Notes for Guidance on preparation of dossiers and study evaluation should be used. The special software package (IUCLID *) must be used for those parts of the dossier for which IUCLID applies.

Annex VI lists the competent authorities of the Member States.

The second Review Regulation contains further provisions as to

- avoiding duplicate vertebrate animal testing,
- joining, replacing or withdrawal of applicants,
- regarding the procedures after submission of dossiers like completeness check and evaluation of the dossiers by the Rapporteur Member State,
- regarding the Commission procedures.

*International Uniform Chemical Information Database

4.2.4 The technical guidance documents

The Biocidal Products Directive describes the requirements necessary for

- the evaluation of active substances
- the authorisation of biocidal products
- the dossier submission including the data requirements.

But to make these processes workable and to ensure a harmonised implementation of the Directive a number of associated Technical Notes for Guidance (TNsG) were elaborated.

Those TNsG and the existing Technical Guidance Document (TGD) on risk assessment for new notified substances and existing substances (revised under inclusion of biocidal substances) explain the information expectations, data use and the evaluation procedures for active substances and the evaluation procedures for active substances and biocidal products.

The guidance documents are published on the website of the European Chemicals Bureau (<http://ecb.jrc.it/biocides/>).

An overview of the existing TNsG and TGD is given in Table 5 and Figure 2.

Table 5 Guidance documents relevant for the implementation of the Biocidal Products Directive.

Short Title	Full Title
TNsG on Data Requirements	Technical Guidance Document in support of the Directive 98/8EC concerning the Placing of Biocidal products on the market – Guidance on data requirements for active Substances and biocidal products, October 2000
TNsG on Annex I Inclusion	Technical Notes for Guidance in support of Directive 98/8/EC of the European Parliament and the Council concerning the placing of biocidal products on the market – Principles and practical procedures for the inclusion of active substances in Annexes I, IA and IB, April 2002
TNsG on Product Evaluation	Technical Notes for Guidance in support of Annex VI of Directive 98/8/EC of the European Parliament and the Council concerning the placing of biocidal products on the market – Common principles and practical procedures for the authorisation and registration of products, July 2002
TGD on Risk Assessment	Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances, Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, April 2003
Environmental Emission Scenarios Documents	Environmental emission scenarios for biocides: Supplement to the methodology for risk evaluation of biocides. Emission scenario document for product type 2: Private and public area disinfectants and other biocidal products (sanitary and medical sector).

Table 5 (Continued)

Short Title	Full Title
	Emission scenario document on drinking water disinfectants (Product type 5).
	OECD Series on Emission Scenario Documents Number 2: Emission scenario document for wood preservatives, part 1 – 4 (Product type 8)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used in paper coating and finishing (Product type 6, 7 & 9)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used as preservatives in the leather industry (Product type 9)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used as preservatives in the textile processing industry (Product type 9 & 18)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used as masonry preservatives. (Product type 10)
	Supplement to the methodology for risk evaluation of biocides. Harmonisation of environmental emission scenarios for biocides used as preservatives for liquid cooling systems (Product type 11)
	Supplement to the methodology for risk evaluation of biocides. Harmonisation of environmental emission scenarios for slimicides (Product type 12).
	Supplement to the methodology for risk evaluation of biocides. Harmonisation of environmental emission scenarios for biocides used as metalworking fluid preservatives (Product type 13)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used as rodenticides (Product type 14)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used as avicides (Product type 15)
	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used in taxidermy and embalming processes (Product type 22)
	Supplement to the methodology for risk evaluation Proposal for the formats of names, parameters, variables, units and symbols to be used in emission scenario documents
Human exposure guidance document	Assessment of human exposures to biocides (first report, 1998)
TNsG on preparation of dossiers and study evaluation	Technical Notes for Guidance on dossiers preparation including preparation and evaluation of study summaries under Directive 98/8/EC concerning the placing biocidal products on the market, December 2002

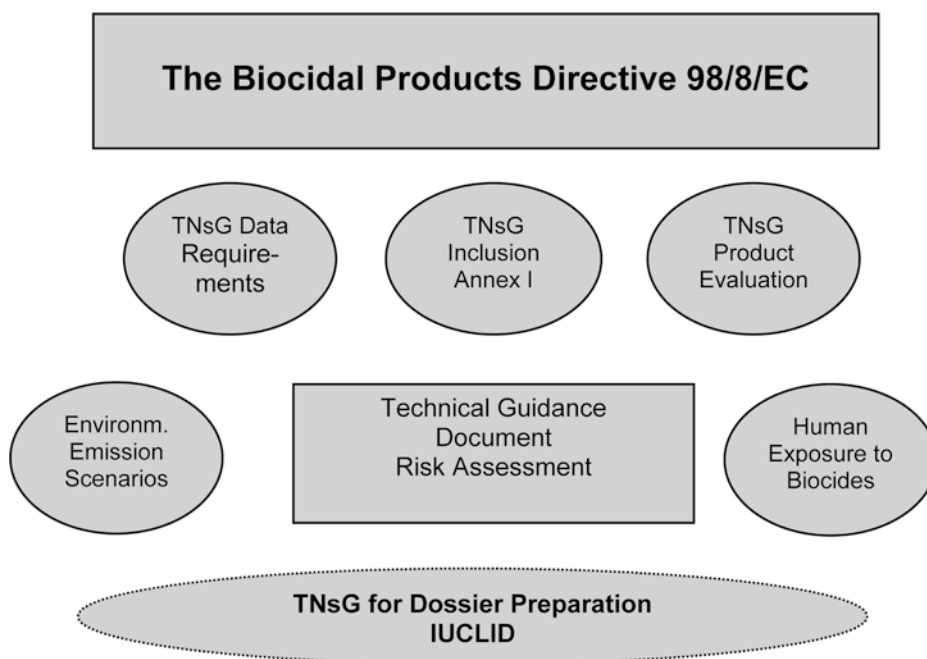


Figure 2 Technical Guidance Documents necessary for Dossier Preparation.

References

1. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal L 230, 19.08.1991, p.1.
2. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. Official Journal L 123, 24.04.1998, p. 1.
3. Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal P 196, 16.08.1967, p. 1 (as amended).

4. Council Directive 87/18/EEC of 18 December 1986 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemicals substances. Official Journal L 15, 17.01.1987, p. 29.
5. Council Directive 88/379/EEC of 7 June 1988 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations. Official Journal L 187, 16.07.1988, p. 14 (as amended).
6. Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work. Official Journal L 374, 31.12.1990, p.1.
7. Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances. Official Journal L 20, 26.01.1980, p. 43
8. Commission Regulation (EC) No 1896/2000 of 7 September 2000 on the first phase of the programme referred to in article 16 (2) of Directive 98/8/EC of the European Parliament and of the Council on biocidal products. Official Journal L 228, 08.09.2000, p. 6.
9. Commission Regulation (EC) No 2032/2003 of 4 November 2003 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, and amending Regulation (EC) No 1896/2000. Official Journal L 307, 24.11.2003, p. 1.

4.3 Protection of health – Microbicides in the environment

C. MACKIE

4.3.1 Introduction

In any registration procedure, risk assessment is now becoming an integral part of the regulatory evaluation dossier and decision-making criteria of regulatory authorities. Most regulatory authorities, particularly in the European Union and the United States, require that a risk assessment should be conducted by industry and this forms the overall decision to the acceptability or otherwise of placing a microbicide product on the market.

A risk assessment, as defined by many international organisations (OECD, 1989; ECETOC 1994; EU TGD, 1996, 2003; EUSES 1996) on an active substance or substance of concern within a microbicide formulation is the evaluation of the following stages:

1. Hazard identification of the substance and subsequent identification of the adverse effects that a substance has an inherent capacity to cause.
2. Dose (concentration) - Response assessment that includes an estimation of the relationship between the dose or level of exposure to a substance, and the incidence and severity of an effect, where appropriate.
3. Exposure assessment (to humans, animals and the environment). This includes an estimation of the concentrations/doses to which human populations (workers, consumers, bystanders and those exposed indirectly via the environment) or environmental compartments (aquatic, terrestrial and air) are or may be exposed.
4. Risk characterisation. This is the final stage of the assessment and is the resulting estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance.

The stages of a risk assessment scheme for substances are presented in Figure 1.

The exposure assessment (left hand side of Figure 1) and the effects assessment (right hand side of Figure 1) taken together lead to a risk assessment that determines the likelihood and severity of adverse effects in the exposed population (human, animal or the environment). This is conducted by comparing the Risk Characterisation Ratios (RCR) such as Predicted Environmental Concentration (PEC)/Predicted No Effect Concentration (PNEC) - PEC/PNEC for the various ecosystems to be protected or the margin of safety (MOS) for exposed human populations.

Each stage of a risk assessment can be a complex process and these will be discussed in more detail in the following sections.

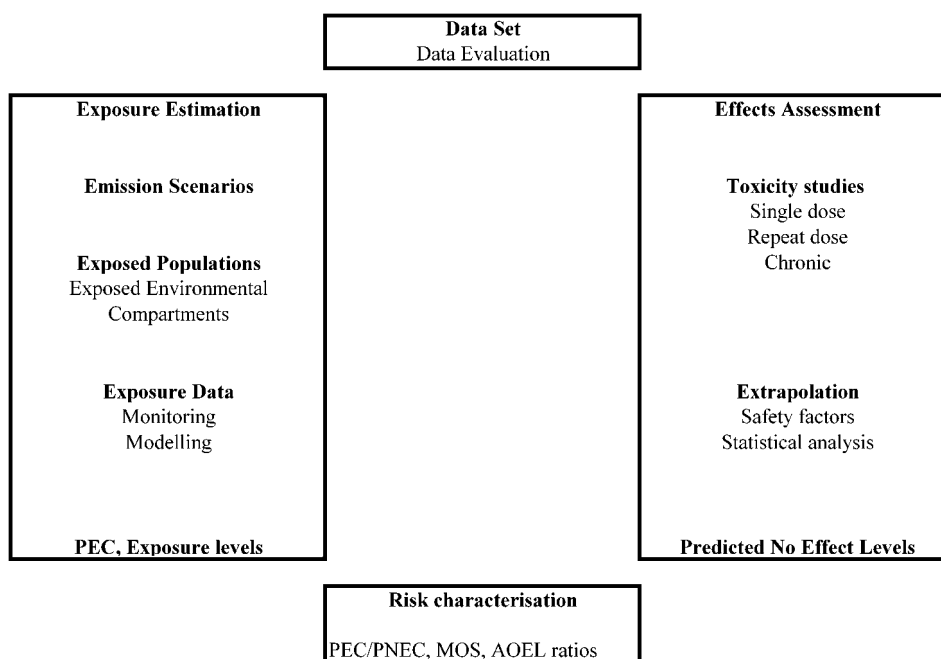


Figure 1 Stages of a risk assessment.

In recent years major developments in risk assessments of biocidal products have taken place within the European Union (EU) as a result of the implementation of the Biocidal Products Directive (BPD) 98/8/EC. This legislation will result in the highest level of testing substances and products of antimicrobial activity and will set the standard for product registration for all suppliers of biocides. Therefore, the risk assessment procedures described within the BPD technical notes for guidance will be discussed in the following sections and compared to other risk assessment procedures worldwide.

4.3.2 Components of a risk assessment

Many aspects of risk assessment are still of a qualitative nature, however, within the EU many models and procedures are now available or under development to allow a more quantitative risk assessment to be conducted. However, it has to be noted that even these quantitative risk assessments are typically conservative in nature and represent a “realistic” worst-case scenario through the lifecycle of a biocidal product, i.e. production, use and disposal.

Under the BPD, no authorisation of a biocidal product will be granted unless a risk assessment is submitted and accepted by the Member State competent authority. Risk assessments are usually separated into three main sections as outlined in the introduction:

- Risk assessment of the physico-chemical properties
- Human health risk assessment
- Environmental risk assessment

4.3.3 Risk assessment of physico-chemical properties

A risk assessment for explosivity, oxidising properties and flammability is required unless none of the product's constituents possess such properties, and, in addition, that on the basis of information available the product is unlikely to present dangers of this kind. Due to the type and nature of the studies conducted under the physico-chemical data requirements section (see Tables 1 and 2 for a list of the required studies on the active substance and 23 product types to fulfill the BPD), a physico-chemical risk assessment on a particular product is usually qualitative and is based solely on the intrinsic hazards of the constituents. Therefore, the outcome of a physico-chemical risk assessment usually relies on the eventual classification of the product for physical and chemical characteristics and this then leads directly to risk management proposals.

Risk characterisation on grounds of explosivity, oxidising properties and flammability

When a biocidal product has been classified as Explosive, Oxidising or Flammable according to National or European law, or where there are other grounds for concern through the intrinsic hazards or intended use of the biocidal product, a risk characterisation has to be conducted. As this is not quantitative, risk characterisation usually leads to a condition on the authorization to manage the risk associated with the product. This can result in restricted use of the product e.g.

- For professional use, engineering controls may be required for the authorization (storage conditions, specifically designed equipment, ventilation requirements)
- Formulation restrictions
- Restriction on use for non professionals
- Disposal restrictions

4.3.4 Risk assessment for human health

A biocidal product can only be placed on the market if the risk assessment confirms that, in foreseeable application including a realistic worst-case scenario, the product presents no unacceptable risk to humans exposed to the substances of the product directly or indirectly through the environment.

The assessment should cover the proposed normal use of the product and treated material if applicable. In addition, the realistic worst-case scenarios should include reasonably foreseeable misuse, such as ingestion by a child, but not accidents or attempted suicides). It should also include relevant production and disposal issues for both the product and treated material. Therefore, the risk assessment should cover the entire the lifecycle of the product.

In the EU, risk assessment methodologies for human health have been described in the Technical Guidance Documents (TGDs) first published in 1996 and adapted in 2003. Initially these guidance documents were produced to support Commission Directive 93/67/EEC on risk assessment for new notified substances and

Table 1 Core Data Requirements for Active Substances under the Biocidal Products Directive (98/8/EC).**PHYSICAL AND CHEMICAL PROPERTIES**

Melting point, boiling point
 Relative density
 Vapour pressure
 Appearance including physical state, colour and odour
 Absorption spectra and a mass spectrum, molar extinction at relevant wavelengths, were applicable
 Solubility in water studies must include the effect on pH (pH5-9) and temperature
 Partition coefficient n-octanol/water including effect on pH (pH5-9) and temperature
 Thermal stability and identity of relevant breakdown products
 Flammability including auto flammability and identity of combustion products
 Flash point
 Surface tension
 Explosive properties
 Oxidising properties
 Reactivity towards container material

ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION

Analytical methods for the determination of pure active substances and, where appropriate, relevant degradation products, isomers, impurities and additives.

Analytical methods in all relevant environmental media (soil, air, water, animal and human body fluids and tissues) including recovery rates and the limits of detection.

TOXICOLOGICAL AND METABOLIC STUDIES

Acute toxicity

oral, dermal, inhalation, skin irritation, eye irritation, skin sensitisation

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study

Repeated dose toxicity

oral, dermal, inhalation

Subchronic toxicity

oral, dermal, inhalation

Chronic toxicity the test is required with one rodent and one other mammalian species

Genotoxicity studies

in vitro mutation study in bacteria

in vitro cytogenicity study in mammalian cells

in vitro gene mutation assay in mammalian cells

If any of the genotoxicity studies gives a positive result, an *in vivo* genotoxicity study is required (bone marrow assay for chromosomal damage or a micronucleus test)

If the *in vivo* genotoxicity test is negative, a second *in vivo* test should be carried out to determine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow.

If the initial *in vivo* test is positive a further study is required to assess possible germ cell effects

If the *in vitro* assays are negative then further testing is normally only required in metabolites of concern are formed in mammals

Carcinogenicity – one rodent and one other mammalian species

Reproductive toxicity

Teratogenicity – one rabbit and one rodent species

Two generations reproduction study

The following data should be presented if available;

Medical data and information on the effects of human exposure (occupational or accidental). (usually not available for new substances).

Medical surveillance data on manufacturing plant personnel

Direct observation – e.g. Clinical cases, poisoning incidents

Health records – both from industry and any other available sources.

Epidemiology studies on the general population

Diagnosis of poisoning including specific signs and clinical tests.

Sensitisation/allergenicity observations

Specific treatments in case of accident or poisoning – first aid measures, antidotes and medical treatment.

Prognosis following poisoning – effects and duration of effects

Summary of mammalian toxicology and conclusions

ECOTOXICOLOGICAL PROFILE INCLUDING ENVIRONMENTAL FATE AND BEHAVIOUR**Fate and Behaviour in the Environment**

Fate and Behaviour in Water

Abiotic Biodegradation

Hydrolysis as function of pH and identification of breakdown products

Phototransformation in water including identity of products of transformation

Biotic Biodegradation

Ready biodegradation

Inherent Biodegradability (where appropriate)

Adsorption and Desorption Screening Test

Ecotoxicological Studies

Acute Toxicity to Fish

Acute Toxicity to Invertebrates

Growth Inhibition Test on Algae

Inhibition to Microbiological Activity

Bioconcentration

Summary of ecotoxicological effects and fate and behaviour in the environment

Table 2 Additional Requirements for Active Substances under the Biocidal Products Directive (98/8EC).**PHYSICAL AND CHEMICAL PROPERTIES**

Dissociation constant

Solubility in organic solvents, including the effect of temperature on solubility

Stability in the organic solvents used in biocidal products and the identity of relevant breakdown products

Viscosity

ANALYTICAL METHODS FOR DETECTION AND IDENTIFICATION**Analytical methods including recovery rates and the limits of detection for residues in/on feed or feedingstuffs and other products where relevant e.g.**

- **Products used as disinfectants in food production and transport,**
- Products used in the food processing industry/catering services
- Products used in paper pulp, paper or any other product intended to be used
- Products placed in or near soils in agriculture/horticulture use
- Residues for fish and shellfish for products in product type 21

TOXICOLOGICAL AND METABOLIC STUDIES

Neurotoxicity studies

Mechanistic studies – any study necessary to clarify effects reported in toxicity studies

Studies on other routes of administration (parenteral routes)

Toxic effects on livestock and pets (usually not required for product types 1, 2, 6, 7, 9, 11, 13, 13, 20, 21 and 22)

Other tests(s) related to the exposure of humans

Identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated food and feeding stuffs

Behaviour of residues of the active substance, its degradation and reaction products and, where relevant, its metabolites on treated or contaminated food or feeding stuffs including the kinetics of disappearance

Estimation of potential or actual exposure of the active substance to humans or animals through food and feeding stuffs and other means

Proposed acceptable residues and the justification of their acceptability

Any other tests related to the exposure of the active substance to humans, in its proposed biocidal products

If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required.

ECOTOXICOLOGICAL PROFILE INCLUDING ENVIRONMENTAL FATE AND BEHAVIOUR(Applicability dependant on core data set results)**Fate and Behaviour in Water**

Biodegradation in seawater

Rate and route of degradation in aquatic systems including identification on metabolites and degradation products

Biological sewage treatment plant studies

Aerobic biodegradation

Anaerobic biodegradation

Biodegradation in freshwater

Aerobic aquatic degradation study

Water/sediment degradation study

Studies on adsorption and desorption in water/sediment systems and, where relevant, on the adsorption and desorption of metabolites and degradation products

Field study on accumulation in the sediment

Fate and Behaviour in Soil

Aerobic degradation in soil, initial study and further studies including the rate and route of degradation, identification of the processes involved, identification of any metabolites and degradation products in at least three soil types

Field soil dissipation and accumulation

Extent and nature of bound residues

Adsorption and desorption in at least three soil types and, where relevant, adsorption and desorption of metabolites and degradation products

Mobility

Fate and Behaviour in Air

Phototransformation in air

Fate and behaviour in air, further studies

Effect on Terrestrial Organisms (if applicable)

Bioconcentration, terrestrial

Effects on other terrestrial non target organisms

Effects on mammals

For product specific data requirements please refer to table

Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. These were adapted in 2003 to take into account the development of current understandings in risk assessment and to include biocidal active substances under the Common Principles (Annex VI) of the BPD.

The risk assessment procedure for human health is usually a quantitative assessment and the stages of the assessment follow those outlined in the introduction of this Chapter. For biocidal products, the risk assessment focuses on the health effects arising specifically from the product itself. However, the majority of the data and endpoints used in the assessment will have been conducted on the active substance(s), substances of concern or other constituents within the product formulation.

Health effects assessment and identification of adverse effects

The product and active substance data requirements under the BPD are detailed in Tables 1–4. In addition to the health effects data requirements, the human health risk assessment should also take into account some physico-chemical data requirements, especially when considering the aspiration hazard of the product.

The following human health effect endpoints need to be considered as part of the product risk assessment:

- Acute, repeat dose and chronic toxicity.
- Irritation/corrosivity.
- Sensitisation.
- Genotoxicity.
- Carcinogenicity.
- Reproduction toxicity.
- Neurotoxicity.
- Other special endpoints where relevant.

Table 3 Core Data Requirements for Biocidal Products under the Biocidal Products Directive (98/8/EC).

PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES

Appearance, physical state, colour and odour
 Explosive properties
 Oxidising properties
 Flash-point and other indications of flammability or spontaneous ignition
 Acidity/alkalinity and, if necessary, pH value
 Relative density
 Storage stability, stability and shelf-life
 Technical characteristics e.g. wettability, persistent foaming, flowability, pourability and dustability
 Physical and chemical compatibility with other products with which its use is to be authorised

METHODS OF IDENTIFICATION AND ANALYSIS

Analytical method for determining the concentrations of the active substance(s) in the biocidal product
 Analytical methods including recovery rates and limits of detection for toxicologically significant components in the following media;

Soil
 Air (required when the substance is volatile or sprayed)
 Water, including drinking water (required for all product types if contamination of water cannot be excluded)
 Animal and human body fluids and tissues
 Treated food or feeding stuffs

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral, dermal, inhalation.
 For products that are intended to be authorised for use with other biocidal products the mixture of products, where possible, shall be tested for acute dermal toxicity and skin and eye irritation.

Skin irritation

Eye irritation

Skin sensitisation

Information on dermal adsorption

Available toxicological data relating to toxicologically relevant non-active substances (i.e. substances of concern)

Information related to the exposure of the biocidal product

ECOTOXICOLOGICAL DATA

Specification of the hazard symbols, indications of danger and relevant risk and safety phrases for the protection of the environment

Prediction of the distribution, fate and behaviour in the environment including timescales

Identification of non-target species and populations which concern arises because of exposure

Identification of measures necessary to minimise contamination of the environment

Identification of foreseeable routes of entry into the environment on the basis of the use envisaged

Describe the predicted receiving environmental compartment and an estimation on how large the amounts released are.

Sources of environmental exposure e.g. production, distribution, storage, mixing including disposal or recovery

Define aquatic recipients e.g. surface water, ground water, estuaries or marine environment

Information on representative measured concentrations or monitoring data which can be used as predicted environmental concentrations in the relevant environmental compartments

Information on the ecotoxicology of the active substance in the product where this information cannot be extrapolated from the information on the active substance itself.

Information required if the composition of or the application technique of the product could influence the degradation and transformation, mobility and adsorption properties or effects on environmental compartments that may alter the conclusions of the risk characterisation.
 A qualitative or quantitative estimate on the possibility of formation of by products of the active substance

Available ecotoxicological information related to ecotoxicologically relevant non active substances e.g. safety data sheets.

Table 4 Additional Data Requirements for Biocidal Products under the Biocidal Product Directive (98/8/EC).**PHYSICAL AND CHEMICAL PROPERTIES**

Surface tension and viscosity
Particle size distribution

METHOD OF IDENTIFICATION AND ANALYSIS

Analytical methods for determining the concentration of the active substance(s) in the product.

TOXICOLOGICAL AND METABOLIC STUDIES

There are no additional toxicological and metabolic data requirements for biocidal products.

ECOTOXICOLOGICAL PROFILE INCLUDING ENVIRONMENTAL FATE AND BEHAVIOUR

Forseeable routes of entry into the environment on the basis of the use envisaged

Testing for distribution and dissipation in soil, water and air

Summary and evaluation of ecotoxicological data

For product specific data requirements please refer to table

The pivotal endpoint to be carried forward to the risk assessment depends on several factors that include:

- Extent and period of exposure
- The exposed population group
- The severity of the endpoint(s)
- The dose-response relationship

From these considerations, the most appropriate No Observed (Adverse) Effect Level (NO(A)EL) is chosen for the risk assessment. From this NO(A)EL the Margin of Safety (MOS) or Acceptable Operator Exposure Level (AOEL) is derived by the incorporation of safety factors. The safety factor used depends on the type of study used to derive the endpoint and the severity of the effect.

Conventionally, a safety factor of 100 is used which incorporates a 10-fold factor for interspecies differences and a further 10-fold factor for inter-individual differences. This safety factor would be appropriate for endpoints relating to e.g. reduced growth rate in a sub-chronic study. However, for endpoints relating to teratogenicity and/or reprotoxicity, the safety factor, depending on the severity of the endpoint could be approximately 200–500 of the NO(A)EL. For carcinogenic substances the safety factor increases yet further to 1000.

It should be remembered that biocidal products can contain two or more active substances or substances of concern that may have additive, synergistic, antagonistic or other combination effects. If additional data are available to allow an assessment of these characteristics, this should form part of the risk assessment on human health even if the data available only allows a qualitative risk assessment to be conducted.

Exposure assessment

An exposure assessment is required for each of the human populations exposed to the biocidal product during production, use and disposal. These populations could include professional users, non-professional users and humans exposed indirectly via the environment. The exposure assessment should make a qualitative assessment of the concentration of the active substance(s) and substances of concern to which a population may be exposed during the lifetime of the biocidal product. Exposure can be categorized into the following groups:

Primary (production or application)

Secondary (non-users, bystanders, people not aware of exposure)

Consumer (exposure via the environment)

For the purposes of the BPD a project was commissioned by DG Environment and conducted by Institutes/organisations from 6 European Member States, together with representatives from industry. The aims of this project were to develop relevant exposure scenarios of humans to biocidal products and to develop operational predictive models for the purposes of authorisation of biocidal products in each of the 23 product types. The report and proposed models from this project is expected to be finalised in mid-2003.

The project has identified several approaches in determining the exposure level for each active substance and substance of concern, these include:

- Models (data) from representative field (real life) exposure studies
- Indicative distribution models
- BEAT model
- CONSEXPO

The indicative distribution models presume that available occupational exposure is log-normally distributed and determines a matrix with 12 cells containing various exposure studies on different use areas. The 12 cells are made up from 3 categories of width of distribution (GSD) – narrow, medium and wide and 4 categories

of typical exposure levels (GM) – low, medium, high, very high. This is a predictive approach to determining the exposure level.

This is shown below in Tables 5 and 6 and taken from the draft report presented to the Technical Meeting on Biocides in 2002.

The project has developed a new model, called BEAT (Bayesian Exposure Assessment Toolkit) and this incorporates the above matrices and seeks to improve and extend their use.

BEAT is only intended for predicted occupational exposure and not consumer exposure. Predictive consumer exposure can be determined using the CONSEXPO model developed by RIVM in the Netherlands and incorporated into EUSES (the European Uniform System for Evaluating Substances). In the USA residential SOPs have been developed for consumer use of antimicrobials and pesticides in the house. These models include:

- Acute, contact with wet surface
- Acute, oral contact with contaminated or treated articles
- Chronic, playing on a treated surface
- Chronic, wearing treated clothing

The results of the quantitative exposure assessment are taken forward to the risk characterisation where they are combined with the results of the effects assessment.

Risk characterisation

For occupational risk characterisation, this should be conducted, in the first instance, without the incorporation of Personal Protective Equipment (PPE) or Respiratory Protective Equipment (RPE). If required, the protective effects of PPE and/or RPE can be used to refine the risk characterisation for appropriate exposure scenarios. If the use of PPE and/or RPE is necessary to reduce the risk to an acceptable level then this would form part of the decision-making process and the appropriate PPE and RPE would have to be included onto the product label.

For use by the general public, where the recommended use of PPE cannot be guaranteed, the incorporation of PPE and/or RPE into a risk characterisation is deemed inappropriate.

In a quantitative human health risk characterisation, the exposure data for relevant use situations is compared to the pivotal NO(A)EL to determine either that the derived Margin of Safety (MOS) is exceeded or that the ratio of the level of exposure/AOEL does not exceed 1. If this is the case, the risk characterisation of the biocidal product should be acceptable.

If the risk characterisation of the biocidal product is not acceptable, based on realistic worst case estimates, then the characterisation must be re-calculated and based on more “typical exposures” and a review of the likelihood of the occurrence and the severity of adverse effects.

The risk characterisation can also be refined by the development of further data to refine the NO(A)EL, AOEL, MOS or exposure levels. Refining the characterisation in this way may also result in an acceptable conclusion.

However, if at the end of the above stages, the level of concern is unacceptable, the competent authority may require risk reduction measures before granting authorization or not allow authorization completely.

Table 5 Example 1 of Indicative Distribution Exposure Models.

Profile	Rate			
	Low	Medium	High	Very High
Narrow	Cabbed orchard spraying TPT (solvent)	Net deployment AF brushing Flea dusting TPT (water)	AF spray	Sheep dipping
Medium		AF mixing and loading		
Wide	Fence brushing	PHI spraying Dipping	Remedial biocide spraying Uncabbed orchard spraying	

AF = Antifouling product

TPT = Timber pre-treatment

Table 6 Calculation of mg/min from Indicative Distribution Exposure Models.

	4mg/min	20mg/min	100mg/min	500mg/min
3.4	9	46	225	1150
5.2	12	60	300	1500
7.1	15	75	375	1850

Table 7 Additional Data Requirements for Different Product Types Ecotoxicity/Fate and Behaviour + date requirements for Active

	Biodegradation in seawater	Anaerobic biodegradation	Adsorption/ Desorption in Soil	*Acute toxicity to fish	*Acute Toxicity to Invertebrates	*Growth inhibition test on algae	*Inhibition of microbiological activity	Effects on reproduction and growth on fish	Bioaccumulation in an appropriate species of fish
1. Human hygiene biocidal products									
2. Private area and health area disinfectants			+ ¹			+	+ ¹ o ¹	+	
3. Veterinary hygiene biocidal products	+ ⁴	+ ²	+ ²	+ ⁴ o ⁴	+ ⁴ o ⁴	+ o ⁴	+ ² o ¹	+	
4. Food and feed area disinfectants									
5. Drinking water disinfectants						+		+	
6. In-can preservatives						+		+	
7. Film preservatives						+		+	
8. Wood preservatives	+ ⁴		+ ⁵	+ ⁴ o ⁴ o ¹⁰	+ ⁴ o ⁴ o ¹⁰	+ ⁴ o ⁴ o ¹⁰	+ ⁵ o ⁵	+	
9. Preservatives for fibres, leather, rubber and polymerised material						+		+	
10. Masonry preservatives			+ ⁵			+	+ ⁵ o ⁵	+	
11. Preservatives for liquid cooling and processing systems	+ ⁴		+ ⁶	+ ⁴	+ ⁴	+	+ ⁶ o ⁶	+	
12. Slimicides	+ ⁴		+ ⁷	+ ⁴ o ⁴	+ ⁴ o ⁴	+ o ⁴	+ ⁷ o ⁷	+	
13. Metal working fluid preservatives						+		+	
14. Rodenticides		+ ²							
15. Avicides		+ ²							
16. Molluscicides	+ ⁴	+ ²	+ ⁵	+ ⁴ o ⁴	+ ⁴ o ⁴	+	+ ⁵ o ⁵	+	
17. Piscicides	+ ⁴				+ ⁴ o ⁴ o ¹⁰	+ ⁴ o ⁴ o ¹⁰			
18. Insecticides, acaricides and other products to control arthropods.	+ ⁴	+ ⁵	+ ⁵	+ ⁴ o ¹¹	+ ⁴ o ¹¹	+ ⁴ o ¹¹	+ ⁵ o ⁵		
19. Repellents and attractants	+ ⁴	+ ⁵	+ ⁵	+ ⁴ o ¹¹	+ ⁴ o ¹¹	+ ⁴ o ¹¹	+ ⁵ o ⁵		
20. Preservatives for food and feedstock									
21. Antifouling products	+			+ ⁴ o ⁴	+ ⁴ o ⁴	+ ⁴ o ⁴		+ ⁴	+ ⁴
22. Embalming and taxidermist fluids									
23. Products for the control of other vertebrates		+ ²	+ ⁸		+ ⁸ o ⁸				

*Core data requirement – always required for more than one environmental compartment (i.e. more than one species) according to exposure

1. For substances to be used as soil or solid waste disinfectants, direct release to the soil is possible.
2. Where releases into manure storage are possible.
3. For use in poultry farms where wild birds are attracted a test with birds is necessary
4. If the substance is to be used in near the coast/offshore or is likely to come in contact with seawater/brackish water the aquatic toxicity tests need to be performed additionally with marine/brackish species.
5. Where high releases to the terrestrial compartment are possible.
6. For substances to be used in cooling systems with an open cooling tower, a high water discharge to air and subsequent deposition onto soil.
7. For inland use of drilling and oil recovery preservatives.
8. If used outside of buildings in the forms of baits, granules or powders, avian toxicity tests are necessary.
9. If used outside of buildings in the forms of baits, granules or powders honeybee toxicity tests are necessary.
10. Aquatic tests to be carried out with freshwater species
11. If the product is to be used as a shark repellent

4.3.5 Risk assessment for the environment

In accordance with the human health risk assessment and physico-chemical assessment, an active substance can only be used in a biocidal product if it is shown to have no unacceptable risks to exposed environmental compartments.

The environmental risk assessment of a biocidal product should cover:

- Production of the biocidal product, if relevant
- Use – this will determine the emission scenarios and environmental compartments of concern
- Disposal – this should include the consideration of fate in soil and water from land fill and the fate in air though incineration

In the EU, risk assessment methodologies for the environment have been described in the TGD's (as for human health). When the TGD's were first published in 1996, the risk assessment guidance covered the freshwater, sediment, soil and air environments. As discussed above these TGD's were amended in 2003

Substances o data requirements for Biocidal Products

Bioaccumulation in an appropriate invertebrate species	Reproduction and growth rate of an appropriate invertebrate species	Effects on sediment dwelling organisms	Aquatic plant toxicity using <i>Lemma</i> spp.	Acute toxicity to earthworms or other soil non-target macro organisms	Acute toxicity to plants	Acute oral toxicity to Birds	Short term toxicity to birds	Effects on avian reproduction	Acute toxicity to honeybees and other beneficial arthropods.	Residue data in fish
	+			+ ¹ o ¹	+ ¹ o ¹					
	+			+ ² o ¹	+ ² o ¹	+ ³ o ³				
	+									
	+									
	+			+ ⁵ o ⁵	+ ⁵ o ⁵					
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	+			+ ⁵ o ⁵	+ ⁵ o ⁵					
	+			+ ⁶ o ⁶	+ ⁶ o ⁶					
	+			+ ⁷ o ⁷	+ ⁷ o ⁷					
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	+			+ ⁵ o ⁵	+ ⁵ o ⁵	+ ⁸ o ⁸ o		+ ⁸ o ⁸		
				+ ⁵ o ⁵	+ ⁵ o ⁵	+ ⁵ o ⁸		+ ⁸	+ ⁵ o ⁹	
				+ ⁵ o ⁵	+ ⁵ o ⁵	+ ⁵ o ⁸		+ ⁸	+ ⁵ o ⁹	
+ ⁴	+ ⁴	+ ⁴	+ ⁴			+ ⁸ o ⁸		+ ⁸		o

and for environmental risk assessment, a new module was included for the first time to cover the marine environment.

Ecotoxicity effects assessment

The product and active substance data requirements under the BPD are detailed in Tables 1–4 and 7. In addition to these data requirements, it is also important to consider the data requirements outlined in the TGDs (2003) where in some case, further data requirements have now been identified, especially in the area of sediment toxicity and marine ecotoxicity.

From these lists of data requirements, Predicted No Effects Concentrations (PNEC's) will need to be derived for:

- Sewage treatment plants exposed during production
- Aquatic environment
- Terrestrial environment

The extent of ecotoxicity data requirements is, to some extent, covered by the exposure and fate and behaviour of the active substance and/or substance of concern. This can be seen in the data requirements table, where extensive data are required for biocidal products that have widespread environmental exposure. Fate and behaviour studies will identify the environmental compartment of concern, e.g. if a substance partitions strongly into sediment from the water column.

PNEC derivatisation for the aquatic environment (Tables 8–9).

From the ecotoxicity studies conducted for freshwater, marine and sediment organisms, the Predicted No Effect Concentration (PNEC) for each compartment can be derived.

The derivatisation of PNECs for the freshwater and marine environments are derived using the following assessment factors. The freshwater PNEC assessments have been well established. However, it has been recognized that the assessment factors proposed by the new marine risk assessment TGD (in development) is highly conservative.

It was considered that a greater species diversity existed in the marine environment and *in lieu* of confirmatory data, it was considered that the distribution of sensitivities to a substance may be broader. Therefore, due to this uncertainty, additional assessment factors have been included in the deriving a PNEC for the marine environment. However, it should therefore be remembered that the final consideration on the assessment factor used in a marine risk assessment will be dependent on the substance, the data available and the number of representative trophic levels covered in the data package.

For the determination of sediment PNECs similar assessment factors are used and in addition, if no data are available, it is possible to calculate a provisional sediment PNEC based on an equilibrium partitioning method. This method uses the PNEC calculated for water for aquatic organisms as described above and the sediment/water partitioning coefficient (TGD, 1996). This method assumes that:

- Sediment-dwelling organisms and water column organisms are equally sensitive to the substance.
- The concentration in sediment, interstitial water and benthic organisms are at thermodynamic equilibrium.

The extent of data to be generated on an active substance used in biocides is dependent on the route of the exposure of the chemical to the environment, the environmental compartments of concern and the need to refine/reduce the assessment factors after the initial risk assessment has been conducted and this will be discussed in more detail below.

PNEC derivatisation for the terrestrial environment.

The same assessment factors are used for the terrestrial compartment as for the freshwater compartment depending on the type of studies available (short term or long term), the number of trophic levels tested. It is recognized that the extent of the data package on the terrestrial environment will be small and typically limited to soil micro-organisms, earthworms and higher plants.

Table 8 Freshwater PNEC Derivatisation.

Information	Assessment Factor
L(E)C50 short-term toxicity tests	1000
NOEC for 1 long term toxicity tests	100
NOEC for additional long-term toxicity tests of 2 trophic levels	50
NOEC for additional long-term toxicity tests of 3 species of 3 trophic levels	10

Table 9 Marine PNEC Derivatisation.

Information	Assessment Factor
L(E)C50 short-term tests from freshwater or saltwater representatives from three taxonomic groups of three trophic levels	10000
L(E)C50 short-term tests from freshwater or saltwater representatives from three taxonomic groups of three trophic levels, + 2 additional marine taxonomic groups	1000
NOEC for 1 long term toxicity test (freshwater or saltwater)	1000
NOEC for 2 long term toxicity tests from freshwater or saltwater representatives from two taxonomic groups of two trophic levels	500
NOEC for 3 long term toxicity tests from freshwater or saltwater representatives from three taxonomic groups of three trophic levels	100
NOEC for 2 long term toxicity tests from freshwater or saltwater representatives from two taxonomic groups of two trophic levels + one long term NOEC from an additional marine taxonomic group	50
NOEC for 2 long term toxicity tests from freshwater or saltwater representatives from two taxonomic groups of two trophic levels + two long term NOEC from an additional marine taxonomic group	10

PNEC derivatisation for sewage treatment plants.

For the determination of PNECs for sewage treatment plants an assessment factor of 10 is currently employed from a study on the inhibition of microbial growth in sewage sludge (TGD, 1996).

Effects of secondary poisoning

As part of the risk assessment, the effects of secondary poisoning have to be considered for both the aquatic and terrestrial environment. Several different characteristics of the substance are taken into account in determining the potential for a substance to be of concern. These characteristics are as follows:

- n-Octanol/water partition coefficient
- Adsorption onto biological surfaces
- Hydrolysis
- Degradation
- Molecular mass

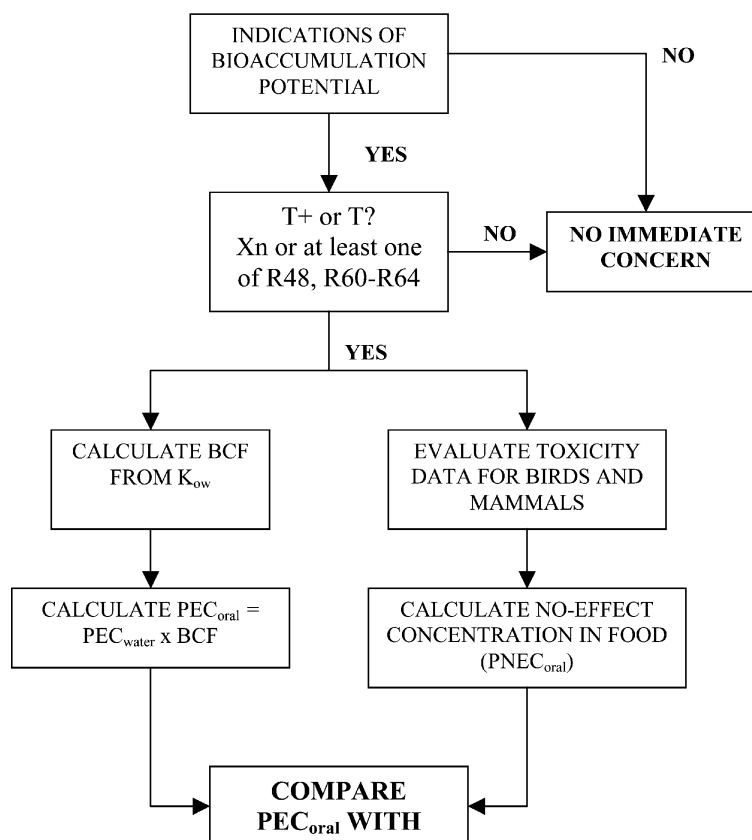
If the results of these tests indicate that the substance has:

- A $\log K_{ow} \geq 3$; or
- Is highly adsorptive; or
- Belongs to a class of substances known to have the potential to accumulate in living organisms; or
- There are indications from its structure;
- Does not hydrolyse or degrade rapidly

There is therefore an indication of bioaccumulation potential.

According to the TGD's an assessment scheme should then be followed to determine the extent of this potential and this is shown schematically below:

Assessment Scheme for Secondary Poisoning



T = Toxic

BCF = Bioconcentration Factor (measured or calculated)

This assessment should be conducted for the aquatic and terrestrial environment e.g.

Water concentration – fish – fish eating bird/mammal

Soil concentration – earthworm – birds/mammals

Exposure assessment

Predicted Environmental Concentrations (PECs) are necessary to determine the level of exposure occurring in each environmental compartment of concern. As the BPD was developing, it was recognized that environmental emission scenarios for many of the 23 product types were not available. As these scenarios are an integral factor in determining the concentration of active substances contained within biocidal products, an EU project was initiated by the European Commission in 2000. The aim of this project was to develop harmonized emission scenarios or standardize existing national scenarios. This project is called EUBEES (EU working group on gathering, review and development of environmental scenarios for biocides) and works very closely with another emission scenario programme set up by the OECD. EUBEES, now in the second stage of the programme has developed and reviewed many of the product types and these are available from the European Chemicals Bureau (ECB) in paper form.

Under the OECD programme the first emission scenario to be developed was for wood preservatives. This is an extensive document covering emissions during application, storage and use of the treated timber and presents scenarios to cover all these areas of use.

The OECD emission scenarios can be presented in a Visual Basic format and as an example, Figures 2 and 3 show the output data for a wood preservative product containing an active substance that leaches from the wood at a rate of $9.5 \text{ mg/m}^2/\text{day}$. The results of these calculations would then be used in the risk characterization stage of the risk assessment as described below.

The wood preservative emission scenario document is nearly complete and the OECD programme is now preparing emission scenarios for antifouling products.

The results of the emission scenario programmes for biocides will eventually be incorporated into a harmonized European-wide model called EUSES (European Uniform System for Evaluating Substances). This allows production to be incorporated into a risk assessment and will calculate PEC values at local, regional and continental levels within the European Union. The extent of the need to calculate regional and continental PEC values will largely be dependent on the results of a local (point source) emission of the active substance. This risk assessment methodology is presented in Figure 4.

The screenshot shows a software window titled "Scenario" with two tabs: "Emissions" (selected) and "Storage". The "Emissions" tab contains the following fields and values:

Parameter	Value
VOLUME(wood-treated) [m3.d-1]:	30
Q(ai) [kg.m-3]:	0.08
F(ai) [-]:	0.01
F(facilitydrain) [-]:	0.0015
Plant: Emissions to Local Air	
Elocal(ai) [kg.d-1]:	.024
Plant: Emissions to Facility Drain	
Elocal(facilitydrain) [kg.d-1]:	.0036

At the bottom of the window, there are two buttons: "Compute Emissions Values" and "Clear Input and Output Values". The status bar at the very bottom reads: "3 - Emission Scenario for Vacuum-Pressure and Double-Vacuum/Low Pressure Processes ()".

Figure 2 Emission from Vacuum Pressure Wood Treatment Process.

Scenario

Storage

Duration of Assessment Period:
☒ Initial TIME1 [d]: 10 ☐ Longer TIME2 [d]:

Emissions at Storage
 FLUX (storage, vac-pres) [kg.m⁻².d⁻¹]: 0.0000095 AREA (wood-exposed) [m².m⁻²]: 11
 AREA(storage) [m²]: ☒ Select or Enter Value: 525 ☐ Compute from Volume:
 TIME(storage) [d]:
 VOLUME(wood-treated) [m³.d⁻¹]:
 VOLUME(wood-stacked) [m³.d⁻¹]:
Computed Value:
Q(leach, storage, time1) [kg]: .5486

Local Concentration in Soil
 V(soil) [m³]: 52.5 RHO(soil) [kg.m⁻³]: 1700
 F(runoff) [-]: 0.5
Clocal(soil, time1) [kg.kg(wwt)-1]: 8.8825

Emission Rate to (Adjacent) Surface Water
Elocal(surfacewater, time1) [kg.d-1]: .0274

3 - Emission Scenario for Vacuum-Pressure and Double-Vacuum/Low Pressure Processes ()

Figure 3 Emission from Stored Wood Following Vacuum Impregnation.

Risk characterisation

For all the exposed environmental compartments, PEC values have to be determined from the manufacture, use and disposal of the active substance(s) contained within the biocidal product. In addition, PEC values may have to be determined for the fate of a treated article.

The PEC values will then be compared to the appropriate PNEC values for the environmental compartment and a PEC/PNEC ratio determined.

If the PEC/PNEC ratio is <1 , then this indicated that there is no concern and that the substance will not pose an unacceptable risk to the environment.

However, if the PEC/PNEC is >1 , this does indicate a risk to one or more environmental compartment and the risk assessment will have to be refined. An assessment can be refined by several means:

- The PNEC can be refined by conducting additional tests that will reduce the uncertainty, and therefore assessment factor incorporated into the PNEC derivitisation.

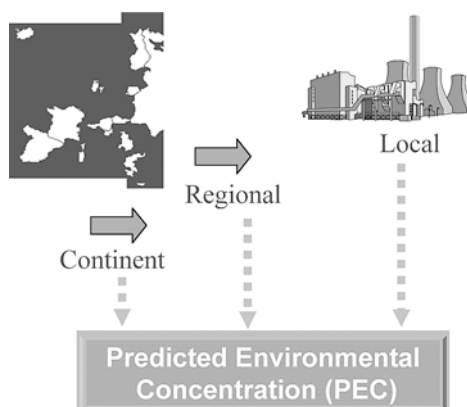


Figure 4 Examples of Generic Environments, as Described in EUSES

- The PEC can be refined by collecting data on emission scenarios to reduce the amount of default values used in the calculation.
- Monitoring data can be collected that will supercede the modeled PEC value(s). However, it has to be noted that this can be an expensive decision and in some cases may not be extensive enough to over rule the modeled data.

4.3.6 Overall conclusions

During a product development of a biocide, a risk assessment for humans, animals and the environment is an integral part of the decisions on the study design and extent of data required in order to obtain registration of a biocidal product.

The risk assessment will determine the acceptable active substance concentrations within a biocidal product and define any restrictions that have to be placed on the manufacture, use and disposal of the substance(s) and product.

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5 Fields of application

5.1 Efficacy of biocides against biofilms

SIMONE SCHULTE, JOST WINGENDER and HANS-CURT FLEMMING

5.1.1 Characteristics of biofilms

The preferred form of life of microorganisms occurs in aggregates. In natural, technical or medical environments, pure cultures are the exception rather than the rule. It is assumed that more than 99% of the microorganisms on Earth live in aggregates (Wimpenny, 2000). Phenomenologically, such aggregates can differ widely, ranging from microcolonies or films on surfaces ("biofilms"), flocs ("floating biofilms") to sludge. The scientific community has agreed to subsume these phenomena under the somewhat inexact expression "biofilms". They all have a common feature: the cells live in close associations at high densities and are embedded in an organic matrix of biopolymers, the so-called extracellular polymeric substances (EPS; Wingender et al., 1999a) which are produced by the organisms themselves. In everyday life, they are known as "slime". Biofilms are the first form of life recorded on Earth, dating back over 3.5 billion years (Schopf et al., 1983) and they also are the most successful form of life. They are found even under extreme conditions such as for example a range of pH-value between 0.5 and 14, temperatures from -5 to 120°C , under strong irradiation as present in primary cooling cycles of nuclear power plants, pressure up to 1,000 bar as encountered on the deep sea floor or shear forces as prevailing at the impact point of water falls (examples compiled by Flemming, 1996). Biofilms develop on virtually any surface in natural soil and aquatic environments, on tissues of plants, animals and humans as well as in man-made technical systems (Costerton et al., 1987; Flemming and Schaule, 1996a). Biofilms also develop on medical devices, causing persistent infections in humans (Costerton et al., 1987). Biofilms are commonly attached to a solid surface (substratum) at solid-water interfaces, but they can also be found at water-air and at solid-air interfaces.

Biofilm systems represent accumulations of microorganisms (prokaryotic and eukaryotic unicellular organisms), EPS, multivalent cations, inorganic particles, biogenic material (detritus) as well as colloidal and dissolved compounds. Polysaccharides are characteristic components of the EPS, but proteins, nucleic acids, lipids and humic substances have also been identified, sometimes in substantial amounts (Wingender et al., 1999a). EPS are considered as key components that determine the structural and functional integrity of microbial aggregates. EPS are involved in the formation of a three-dimensional, gel-like, highly hydrated and locally charged (often anionic) biofilm matrix, in which the microorganisms are more or less immobilized. EPS are responsible for binding cells and other particulate materials together (cohesion) and for anchoring biofilms to the substratum (adhesion). They create a microenvironment for sessile cells which is conditioned by the chemical nature of the EPS matrix. In general, the proportion of EPS in biofilms can vary between roughly 50 and 90% of the total organic matter (Christensen and Characklis 1990; Nielsen et al., 1997). Figure 1 shows a conceptual view of a biofilm as reconstructed from horizontal sectioning by confocal laser scanning microscopy (Costerton et al., 1994).

An important aspect of biofilms is their structural and functional heterogeneity with pores and channels. For example, active heterotrophic bacteria may consume oxygen at a rate which is faster than its diffusion rate. In such a case, even in an aerobic system an anerobic zone will develop underneath an aerobic zone and give rise to the development of organisms which would not be expected in an aerobic system. This can lead to the growth of sulphate reducing bacteria at the base of biofilms on the walls of fully aerated water reservoirs (Christensen and Characklis, 1990).

Although the details of biofilm development processes vary according to species general distinct developmental steps have been recognized in bacterial biofilm formation (for review, see O'Toole et al., 2000; to Dunne, Jr., 2002). These include the initial attachment to a surface, followed by the formation of microcolonies, and finally the maturation of microcolonies into an established biofilm, which is maintained in a stable form by the EPS matrix. Environmental parameters (e.g., nutrient availability, osmolarity, pH, oxygen tension, temperature) seem to determine the transition from planktonic life to growth on a surface. According to Donlan and Costerton (2002), bacteria form biofilms preferentially at high-shear locations in natural and industrial systems; thus, planktonic cells can adhere to surfaces and initiate biofilm formation under turbulent flow in the presence of shear forces exceeding Reynolds numbers of 5,000 (Donlan and Costerton, 2002). Mature biofilms are highly viscoelastic, and are stronger and more resistant to mechanical breakage when grown in high-shear environments compared to biofilms formed under low-shear conditions.

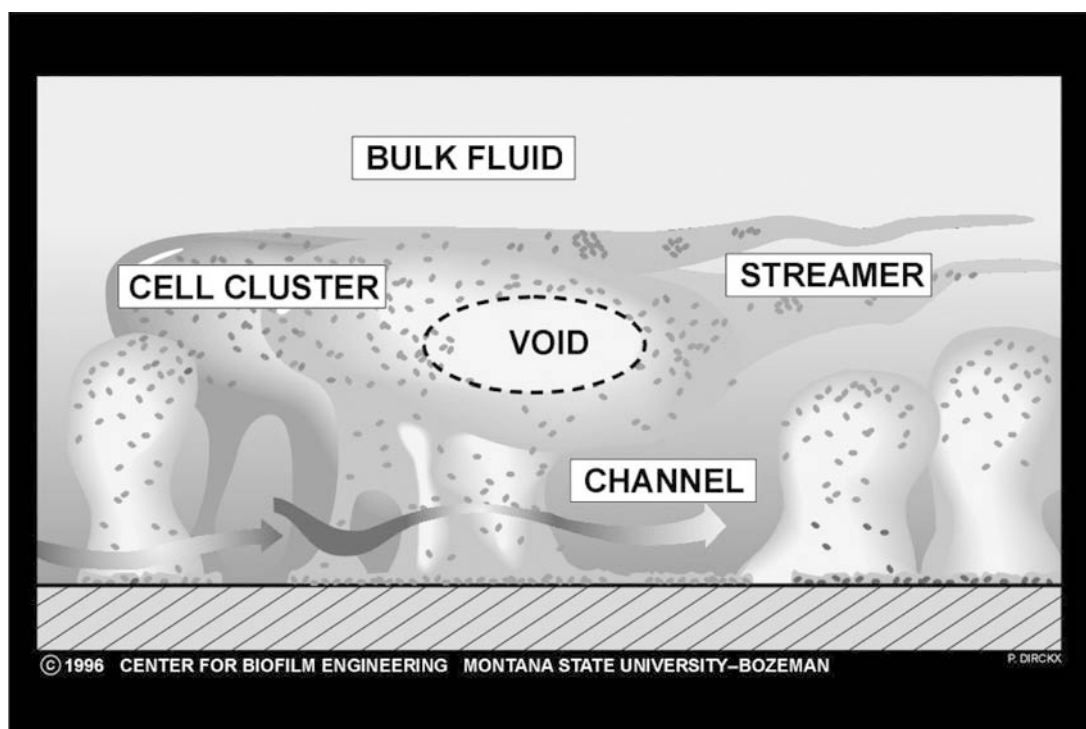


Figure 1 Vertical view of a biofilm (after Costerton et al., 1994).

In recent years, it has become obvious that the biofilm mode of growth is associated with a specific expression of genes and altered growth rates. To take into consideration the adoption of characteristic biofilm phenotypes by planktonic bacteria, a modern definition of a biofilm has been given by Donlan and Costerton (2002), who described a biofilm as “a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of EPS that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription”.

Biofilms can have both beneficial and detrimental effects. Beneficial functions include the degradation and turnover of organic matter in natural soil and water environments, or the purification of raw sewage in wastewater treatment plants. Biofilms can have detrimental effects in the human environment when occurring in the wrong place and/or the wrong time. This undesired development of biofilms on surfaces is referred to as *biofouling* (Flemming, 2002). Biofilms can be involved in the destruction of the materials they colonize; these processes are described as *biocorrosion*, *microbially influenced corrosion* (MIC) or *biodeterioration* (Dowling et al., 1991). The damage of biofouling is very difficult to assess, but even crude estimates amount to many millions of \$US every year in industrialized countries (Flemming, 1996).

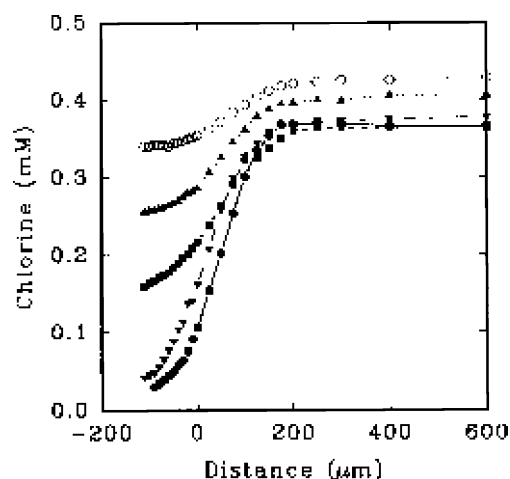


Figure 2 Transient profiles measured at high chlorine concentrations (0.28 to 0.42 mM) in a dual-species biofilm (*P. aeruginosa* and *klebsiella pneumoniae*). The chlorine profiles were recorded 3 (●), 15 (▼), 30 (■), 45 (▲), and 60 (○) min after the start of the chlorine dosing. Zero on the x axis corresponds to the surface of the cell cluster as estimated visually at the start of the experiment. (in DeBeer et al., 1994b).

Biofilms are involved in all kinds of biofouling (Kent, 1988; Flemming, 2002). When biofilms develop on ship hulls, in industrial pipe systems and other fluid flow systems, they increase frictional resistance due to the viscoelastic properties of the EPS matrix. This may lead to a substantial pressure drop and increase in energy consumption or to a reduction of speed of vessels. Christensen and Characklis (1990) assessed that a biofilm of 50 μm thickness, calculated as rigid roughness can lead to a speed loss of 5–12% of a vessel. In cooling water systems, they cause increase in resistance to heat energy transfer, increase in fluid frictional resistance, or acceleration of metallic corrosion. The performance of a heat exchanger can be significantly decreased by a biofilm because only diffusional heat transfer is possible (Characklis, 1990b). Convective heat transfer as achieved by tangential flow of water across the heat exchanger surface is hampered by the biofilm which then acts as an insulating layer for convection. In some cases, biofilms result not only in the unwanted accumulation of biological material on surfaces, but also promote the precipitation of minerals, especially calcium carbonate. This leads to mixed biological and non-biological deposits (Heath *et al.*, 1992) which are particularly difficult to remove. Calcium precipitation is an important aspect in scaling of surfaces e.g., on heat-exchanger surfaces, on separation membranes, on ship hulls and oil rigs.

In drinking water distribution systems, biofilms provide a habitat for potentially pathogenic microorganisms, which can be released into the water and pose a health hazard to the consumer; they may also be the cause of organoleptic problems. Figure 3 shows a scanning electron micrograph of a biofouling case in which the biofilm developed on a synthetic rubber coating of a valve in a drinking water system, harbouring hygienically relevant organisms such as *Citrobacter* (Kilb *et al.*, 2003). The material between the cells is EPS embedding the biofilm organisms. Due to dewatering, the EPS collapsed but it is still apparent that this material has integrated the bacteria in its matrix.

Contamination of ultrapure water by biofilm bacteria interferes with the production of microchips. In the beverage and food industry, biofilms on equipment surfaces can be involved in the spoilage of the products. Biofilms can also be important in the clinical setting, when they develop on medical devices (Donlan, 2001) such as implants, catheters, contact lenses, leading to life-threatening infections.

The above-mentioned examples show clearly that many problems in technical water systems are caused by biofilms and not by planktonic cells. Thus, countermeasures against biofouling must be directed against surface-attached biofilms. These measures should include the detection, monitoring, removal, prevention or at least control of biofilm formation (Flemming, 2000). A variety of sanitation measures for the treatment of biofouling exist such as regular cleaning using physical methods (e. g., rinsing, brushing, ultrasonic treatment),

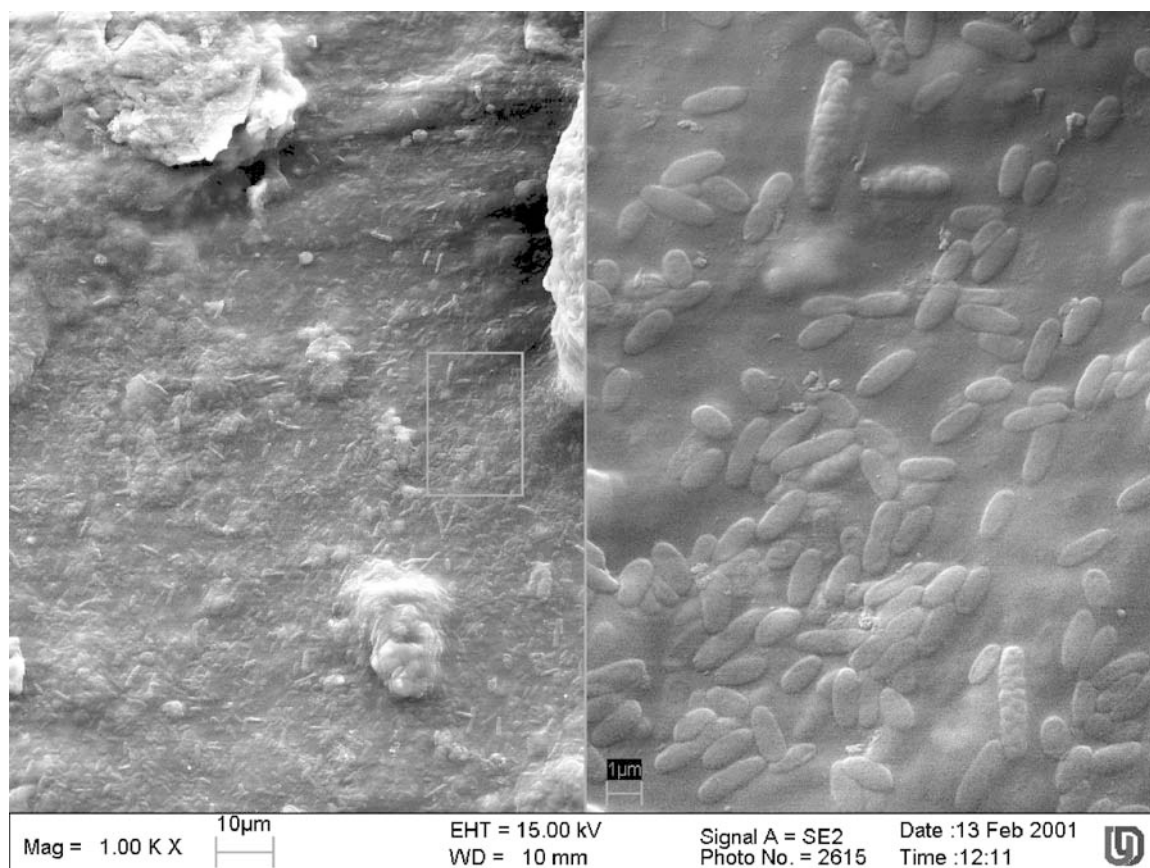


Figure 3 SEM micrograph of a biofilm on a synthetic rubber coating of a drinking water valve (from Kilb *et al.*, 2003).

application of chemical agents (oxidants, alkali, surfactants, enzymes, complexing substances, dispersants) to kill and detach biofilm organisms, limitation of nutrients to minimize microbial growth, etc. (Flemming and Schaule, 1996b; Flemming, 2002).

As indicated in these examples, unwanted biofilms occur in very different fields and are approached rather individually. In most cases, there is one common misunderstanding: the problems are considered as a kind of “technical disease” in terms of a medical analogy which is reflected in the use of the medically defined term “disinfection”. While the killing of invading microorganisms will be the cure of choice in living organisms, this is in most cases not the case for technical systems. In contrary to an immune system, which eliminates dead microorganisms, dead biomass in a technical system will stay in place and provide nutrients for cells which enter the system later. Therefore, it is most important to distinguish between killing and cleaning, with cleaning being at least equally important as killing. Some biocides may have an additional cleaning effect but most do not. In such cases, a disinfection will not lead to a solution of the problems but sometimes lead to rapid regrowth and a “saw-toothed curve” of biofilm development with a rising baseline (Flemming, 1991). This is frequently observed in practice and represents the cause of failures of anti-fouling measures using disinfectants and biocides, but it is usually not published. Highlighted by this background, we will separately address biocidal vs. cleaning effects in the following discussion of various biocides.

5.1.2 Definitions of antimicrobial agents

It is useful to define the different terms concerning antimicrobial agents applied to treat biofouling problems.

A *biocide* is a chemical agent that inactivates living organisms, pathogenic and nonpathogenic. In the context of the application of biocides as antifouling agents, Cloete et al. (1998) described biocides as “antimicrobial agents employed in various spheres of human activity to prevent, inhibit or eliminate microbial growth”. In the control of biofouling, the primary aim of biocide application is usually to reduce microbial numbers on a surface in order to restore or maintain the proper functioning of a technical system. A wide variety of oxidizing and non-oxidizing inorganic and organic biocides are known; they are commonly used in commercial formulations to control biofouling in industrial systems or in clinical environments. A number of biocides can also have a cleaning function; in this context, cleaning means the physical removal of unwanted biofilm material from a surface.

Disinfectants and antiseptics are biocides or products that are primarily used to inhibit or destroy hygienically relevant microorganisms; they are used to prevent infection, i.e., transmission of pathogenic or potentially pathogenic microorganisms. As for the treatment of surface-attached microorganisms, disinfectants are applied to inanimate objects or surfaces, whereas antiseptics are used to inactivate microorganisms in or on living tissues (Block, 1991a; McDonnell and Russell, 1999). In biofouling treatment, biocides can be used as disinfectants, when the aim is to selectively inactivate disease-causing microorganisms, for example *Legionella* bacteria in biofilms of cooling water systems and hot water systems, or opportunistic microorganisms on the surfaces of medical devices.

Antibiotics [II,20.11]* are naturally occurring organic substances produced by certain microorganisms (bacteria and fungi), which inhibit the growth of other organisms, generally at relatively low concentrations. Antibiotics do not play an important role in their application in technical systems, but are used therapeutically to inactivate planktonic and biofilm organisms in the treatment of infectious diseases.

In general, biocides have a broad spectrum of activity and have multiple targets; an exception are antibiotics, which tend to have specific cellular targets. The response of microorganisms to biocides depends on the type of organism, the biocide itself, and the concentration. It is not easy to elucidate the exact mechanism of action of a biocide. The reason for this is that more than one cell constituent may be affected, and consequently the problem is to distinguish the primary effect from the secondary effects, which may, however, contribute to cell death. Target structures of biocides include the cell wall, cytoplasmic membrane and ribosomes of vegetative cells, the coat and cortex of bacterial spores, and the envelope and capsid of viruses; biocides can react with macromolecules such as proteins (structural proteins, enzymes), nucleic acids and polysaccharides. The results are the disruption of membranes with leakage of intracellular components, and the destruction of many cellular functions, including replication, transcription, protein synthesis, and general metabolism. A number of recent reviews give more detailed information about the mechanisms of action of biocides (Cloete et al., 1998; McDonnell and Russell, 1999). See also Part One, Chapter 2.

*see Part Two – Microbicide Data

5.1.3 Resistance of biofilm organisms to biocides

Biofilms are known to exert enhanced resistance to biocides (LeChevallier et al., 1988 a,,b). There are different mechanisms of resistance, depending upon the biocide, the biofilm and the environmental conditions. Some of the major factors are discussed in the following sections.

5.1.3.1 Influence of abiotic factors

In adverse environments, microorganisms in biofilms are supposed to have a survival advantage over planktonic cells (Marshall, 1985). Under practical conditions, the success of biocide treatment depends not only on the biological resistance properties of the microorganisms *per se*, but is also determined by other factors such as choice and type of biocide, treatment regime, and a number of different environmental parameters (Cloete and Brözel, 2002). Since several of these factors can interact antagonistically or synergistically, it cannot always be determined unequivocally to which extent the resistance properties of one organism actually contribute to their survival.

The influence of abiotic factors on biocide efficacy has been largely studied on planktonic populations (Bessems, 1998), but can be expected to be also relevant to biofilms. For example, the enhanced efficacy of many biocides with increasing *temperature* has been described for the treatment of *Pseudomonas aeruginosa* biofilms; thus, a formulation of peracetic acid and hydrogen peroxide caused an increase in killing when the temperature was increased within the temperature range of 10°C to 50°C (Blanchard et al., 1998).

The *incorporation of abiotic particles* such as kaolin, calcium carbonate or iron-containing corrosion products into biofilms can result in a reduced biocide efficacy as has been shown for chlorine and monochloramine (LeChevallier, 1991; Srinivasan et al., 1995). This effect has important implications, since biofilms can be dominated by particulate matter in industrial systems such as cooling towers or water pipelines.

In most practical situations, biofouling is caused by submerged biofilms. Therefore, *hydrodynamic conditions* have to be taken into consideration for biocide application. Flow velocity can have a marked effect on the killing and detachment of biofilms by biocides in aquatic systems. For example, Bott (1998) mentioned that, after 1 hour in the presence of a chlorine concentration of 4.7 mg/L, the removal of an established biofilm was approximately 20% higher at a flow velocity of 1.27 m/s compared to a flow velocity of 0.86 m/s. Turbulent flow improved the inactivation of sessile bacteria (*P. fluorescens*) on metal coupons by 0.15 mg/L ozone (contact time of 1 hour), resulting in a decrease of about one order of magnitude in cell density compared to the killing of the same biofilms under static conditions (Viera et al., 1999a, b). The transition from laminar to turbulent flow was accompanied by a 99.7% increase in the inactivation of biofilm bacteria (*P. aeruginosa*) during treatment with 15 mg/L peracetic acid (Blanchard et al., 1998). Possible causes for enhanced biocidal efficacy under high flow rates are thought to be improved mass transfer and/or a higher shear at the biofilm/water interface, promoting the access of the biocide into the biofilm.

5.1.3.2 Enhanced resistance of biofilms

In practical situations, biofilms are often difficult to eradicate and prove recalcitrant to the application of biocides. According to Gilbert et al. (2001) biofilms are 10 to 1 000 times less susceptible towards a wide variety of different antimicrobial agents than are the corresponding planktonic cells. This phenomenon is supposed to be due essentially to various resistance mechanisms that are associated with the biofilm mode of growth. Recent reviews have been published, covering different aspects of resistance of biofilms to antimicrobial agents (Brown and Gilbert, 1993; Morton et al., 1998; McDonnell and Russell, 1999; Gilbert et al., 2001; Lewis, 2001; Mah and O'Toole, 2001). In many cases the exact mechanisms of biofilm resistance are still unclear and are only beginning to be elucidated. Here, a short summary is given on the known resistance mechanisms of established biofilms that undergo treatment with biocides.

In the context of this chapter, resistance is defined as the ability of a microorganism to grow in the presence of elevated levels of an antimicrobial substance or to survive the treatment with an antimicrobial substance. Under practical conditions, elevated levels of a biocide are those which are higher than the concentrations usually used for the killing or control of microbial contaminations in a specific application. It must be pointed out that most biofilm susceptibility studies in the laboratory and in practical situations have been performed on already established biofilms, analyzing the survival and persistence of biofilm cells, while the efficacy of biocides on biofilm growth is rarely considered. As to inhibition of growing cells, biofilm organisms have been described which do not grow better than planktonic cells in the presence of many antimicrobials (Lewis, 2001), indicating that biocides may prevent or at least delay and control the colonization and biofilm formation on surfaces. However, mature biofilms exhibit an increased resistance to killing by biocides.

Two major types of microbial resistance can be distinguished: intrinsic and acquired resistance. Intrinsic (innate) resistance refers to a natural chromosomally controlled property, including physiological adaptation,

that is specific for a certain type of microorganism. Acquired resistance may be due to mutations with subsequent selection of resistant mutants from the population which has been exposed to the biocide, or it may result from the uptake of plasmids or transposons which confer resistance to biocides (Morton, 1998; McDonnell and Russell, 1999). Formation of a biofilm can be regarded as a physiological (phenotypic) adaptation, and thus represents an intrinsic mechanism of microbial resistance to biocides. At present, it is not known if acquired resistance is of importance in biofilm resistance. It can be speculated that the high cell densities in biofilms may enhance the probability of spontaneously resistant mutants to be selected on exposure to sublethal concentrations of biocides; in addition, high cell numbers may promote horizontal transfer of genes expressing resistance to biocides (Davey and O'Toole, 2000).

Many different mechanisms of biofilm resistance are discussed in the literature, reflecting the different ways of biofilm organisms to withstand biocides. These mechanisms include physical and chemical diffusion-reaction barriers in the biofilm restricting biocide penetration of the biofilm, slow growth rate of biofilm cells due to nutrient limitation, activation of general stress response genes, the emergence of a biofilm-specific phenotype, and the presence of persister cells.

5.1.3.3 *Transport limitation by reaction-diffusion interaction*

One of the first explanations for the increased resistance of biofilm organisms to biocides was diffusion limitation (Costerton et al., 1987). The reported resistance often refers to an increased resistance of the established biofilm population as a whole to the killing of the applied biocide. However, when biofilm bacteria are dispersed (removed from the intact structure of the biofilm), the susceptibility of these suspended organisms may be enhanced or equal to that of planktonically grown cells. This effect has been reported for biofilm bacteria treated with chlorine and chlorosulfamate (Gilbert et al., 2001). Griebe et al. (1994) found that homogenized *P. aeruginosa* biofilms were significantly more sensitive to chlorine compared to intact biofilms, whereas monochloramine showed decreased killing against homogenized biofilms. When biofilms of *P. aeruginosa* were dispersed, their sensitivity to quaternary ammonium compounds was strongly enhanced, almost to the level of planktonically grown cells; however, recovery of sensitivity was poor in suspended biofilms of *Staphylococcus aureus* (Campanac et al., 2002).

These observations indicate that, depending on the type of biocide and species-specific biofilm formation, the resistance of single biofilm cells within the bulk biofilm population is not necessarily changed compared to planktonic cells. In these cases, resistance of the biofilm population is brought about by the characteristic spatial arrangement of cells within the EPS matrix inherent to the biofilm mode of growth. Since the development of a three-dimensional biofilm structure depends on the presence of EPS, the production of these molecules can be considered as part of a physiological adaptation process of the whole population during biofilm formation with the consequence of enhanced resistance to antimicrobial treatments (Hentzer et al., 2001).

It has been suggested that the EPS matrix presents a potential barrier which delays or prevents biocides from penetration into the biofilm and from reaching target organisms in all parts of a biofilm. The plausible assumption was that mass transfer of antimicrobial agents to microorganisms might be restricted by the EPS matrix, acting as a diffusion barrier or by interaction of the biocide with matrix components. However, no significant diffusion limitation was observed for small, non-reacting molecules the size of biocides. They were shown to diffuse freely in the biofilm matrix and to penetrate biofilms of up to 1 mm in thickness relatively quickly, within seconds or minutes (Stewart, 1996, 1998). In addition, biofilms have been shown to be structurally heterogeneous with pores and channels, allowing for convective flow to a small extent throughout the biofilm, so that access of biocides is not necessarily limited to most parts of a biofilm.

Another explanation for reduced biocide penetration into biofilms is the interaction between biocide and biofilm constituents, including cells and EPS; a result would be depletion of the antimicrobial compound in the biofilm interior. The underlying mechanisms may be chemical reactions of the biocide with, or sorption to, the biofilm components or the enzymatic degradation of the biocide, resulting in a restricted penetration of the biocide into the biofilms (reaction-diffusion interaction mechanism).

A large number of studies has been performed on the chemical interaction between biofilms and oxidizing biocides [II, 21] such as chlorine (sodium hypochlorite) and hydrogen peroxide, which have wide-spread application in industrial and clinical settings. Using a chlorine-sensitive microelectrode, chlorine penetration into biofilms containing *P. aeruginosa* and *Klebsiella pneumoniae* was found to be a function of simultaneous diffusion and reaction in the biofilm matrix (de Beer et al., 1994b; Figure 2); chlorine concentrations were only 20% or less of the concentration in the bulk liquid over a 1 to 2 hour period. The shape of the chlorine profiles indicated chlorine consumption within the biofilm matrix. Similar results were obtained using an artificial biofilm system consisting of *P. aeruginosa* entrapped in alginate and agarose gel beads (Xu et al., 1996).

EPS are considered as reactive compounds for biocides. However, this depends on the nature of the antimicrobial agent and the composition of the EPS, and whether the EPS matrix constitutes a penetration barrier and protective structure for biofilm organisms. For example, in mucoid strains of *P. aeruginosa*, slime formation afforded protection against chlorine, but not against hydrogen peroxide (Wingender et al., 1999b). It was

demonstrated that chlorine chemically reacted with the exopolysaccharide alginate (major EPS component of mucoid *P. aeruginosa*) under rapid chlorine consumption and release of trihalomethanes; in contrast, hydrogen peroxide did not interact with the alginate (Wingender et al., 1999b).

Aldehydes [II, 2.] react with amino acid residues of proteins, which may be a substantial fraction of the EPS. The reaction of glutaraldehyde with extracellular proteins in *P. fluorescens* biofilms was supposed to reduce the antimicrobial action of the biocide (Pereira and Vieira, 2001).

Cationic biocides may be immobilized by binding to negatively charged microbial exopolysaccharides; thus, cationic biocides including biguanides [II, 18.3.] and quaternary ammonium compounds [II, 18.1.] interact with EPS molecules by electrostatic interactions (Morton et al., 1998), restricting permeation of the biocide through the biofilm. This mechanism may also apply to certain antibiotics; for example, their interaction with the polyanionic exopolysaccharide alginate has been supposed to contribute to retarded penetration into thick biofilms (Ishida et al., 1998).

Biofilm organisms produce a large number of different degradative enzymes which accumulate in the biofilm matrix (Wingender and Jaeger, 2002) and may provide a potential for the enzymatic degradation of biocides. For example, catalase enzymes neutralize hydrogen peroxide by disproportionation of the biocide into oxygen and water. In biofilms of *P. aeruginosa*, a constitutively expressed catalase (KatA) has been shown to protect the bacteria by preventing full penetration of hydrogen peroxide into the biofilms (Stewart et al., 2000). A second catalase (KatB) was induced in biofilms as an adaptive response to sublethal amounts of hydrogen peroxide; this effect was supposed to be an acquired resistance mechanism for biofilm protection when initial biocide levels were sublethal (Elkins et al., 1999). Additional evidence for the importance of catalases in the protection of biofilm-forming bacteria comes from the observation that catalase-deficient mutants revealed enhanced susceptibility to hydrogen peroxide (Elkins et al., 1999; Wingender et al., 1999b). Another example of enzymatic inactivation is the degradation of some antibiotics by β -lactamase enzymes in bacterial biofilms (Giwerzman et al., 1991). Calculation of β -lactam penetration reveals that this catalytic reaction can lead to severe antibiotic penetration failure (Stewart, 1996).

All of these observations indicate that biofilm constituents in the outer layer of biofilms can interact with certain biocides and remove them in such quantities and at sufficiently high rate to protect more deeply embedded microorganisms (Xu et al., 1996). Practical consequences are that complete inactivation of biofilm organisms can only be expected if large enough quantities of biocides are applied over a sufficiently long period of time or even continuously, ensuring complete penetration of the biofilm. Subinhibitory levels of biocides may induce the formation of EPS components (Leyval et al., 1984; Elkins et al., 1999), which will be involved in enhanced survival due to the reaction-diffusion interaction mentioned above. However, the reaction-diffusion limitation mechanism based on interactions between biocide and EPS components cannot always completely account for biofilm resistance, and other physiological mechanisms must be expected to be implicated in resistance.

5.1.3.4 Slow growth rate and general stress response

In many biofilm environments, microorganisms live under conditions of nutrient limitation (Marshall, 1985). Biofilms preferentially grow in oligotrophic conditions, so that supply of nutrients may be limiting; competition for nutrients among biofilm organisms which are typically present at high cell densities may further promote nutrient limitation. In addition, nutrient access to all parts of a biofilm may be incomplete due to consumption of the biocide. This is supposed to be one reason for the spatial heterogeneity of physiological activity in biofilms (Huang et al., 1995; Xu et al., 2000; Mah and O'Toole, 2001).

Growth rate has been implicated in susceptibility to biocides. At slow growth rates, susceptibility to certain antimicrobial agents may be diminished. Distinct regions of faster and slower growth have been observed throughout the same biofilm (Wentland et al., 1996). Bacteria located deep in the biofilm may experience a nutrient-deficient environment, so that in these parts of the biofilm bacteria grow slowly or not at all (Huang et al., 1995). Nutrient limitation may switch cells into a dormant and thus protected phenotypic state. A consequence of nutrient gradients is the development of physiological heterogeneity within a biofilm, which is also reflected in a differential response of individual cells within the same biofilm. For example, the pattern of respiratory activity in a *K. pneumoniae* biofilm exposed to monochloramine showed that the bacteria near the biofilm-bulk liquid interface lost activity first (Huang et al., 1995). Many antibiotics only kill growing cells or are more effective against rapidly dividing cells, so that absence or decrease of growth may be a reason for biofilm resistance to these types of antibiotics. In this respect, biofilms resemble stationary-phase planktonic cells in batch cultures, which are also characterized by reduced growth and are less susceptible to antimicrobial agents compared to planktonically grown logarithmic cells (Spoering and Lewis, 2001).

Based on these observations, it can be expected that the efficacy of biocides can vary greatly, depending on the location and the physiological state of the target cells within a biofilm.

As pointed out, slow growth seems to be responsible for a certain level of resistance, but there is evidence that it only adds protection in addition to other mechanisms (Mah and O'Toole, 2001). It has been suggested that

slow growth is only one aspect of a general stress response triggered by microbial growth within a biofilm. The result would be physiological changes, which can render biofilms more resistant to various environmental stresses, including heat and cold shock, pH changes or the presence of antimicrobial agents. The basis for stress-resistant phenotypes are changes in gene expression and regulation. For example, there is some evidence that alternate sigma factors have a role in biofilm resistance to certain oxidative biocides. Thus, in thin biofilms of *P. aeruginosa*, the sigma factors RpoS and AlgT were shown to be involved in resistance to hydrogen peroxide, but not to monochloramine (Cochran et al., 2000); in thick biofilms, the sigma factors did not contribute significantly to hydrogen peroxide resistance, suggesting a transient role of the sigma factors in this biofilm system.

5.1.3.5 Role of biofilm-specific phenotype

Evidence is accumulating that the process of attachment to surfaces and growth in a biofilms is associated with the activation and repression of genes, resulting in a biofilm-specific phenotype of the microorganisms within a biofilm community. It is assumed that this process includes the expression of a biocide-resistant phenotype in all or a subset of the biofilm cells (Mah and O'Toole, 2001). Induction of this phenotype may be caused by nutrient limitation, environmental stress, exposure to sublethal amounts of biocides, high cell density or a combination of these factors.

Induction or upregulation of exopolysaccharide production is a phenotypic characteristic of surface-attached bacteria; for example, in *P. aeruginosa*, the transcription of key genes involved in the biosynthesis of alginate is induced soon after the bacteria attach to a solid surface (Davies, 1999). These regulatory processes may contribute to biocide resistance, indirectly by mediating the development of the three-dimensional biofilm architecture and providing protected niches for biofilm organisms, or directly by representing target molecules for the interaction and quenching of biocides as described above.

Biofilm formation is influenced by the phenomenon of quorum sensing, which is the regulation of gene expression-based cell-cell communication, frequently in response to population density (for recent review, see Miller and Bassler, 2001). This kind of bacterial communication is mediated by low-molecular-weight diffusible signal molecules called autoinducers. Among them, N-acylhomoserine lactones (AHLs) are the best studied class of signal molecules. Extracellular accumulation of AHLs above a critical threshold level results in transcriptional activation of a range of different genes with concomitant expression of new phenotypes. Thus, it can be expected that quorum sensing may influence biocide resistance, either indirectly by influencing the formation of the biofilm (Davies et al., 1998), or by regulating genes whose products are directly involved in resistance.

Other possible phenotypic changes that are discussed include decrease of membrane permeability due to alterations in membrane compositions or the upregulation of multidrug efflux pumps that could extrude biocide molecules from the cell interior (Gilbert et al., 2001; Mah and O'Toole, 2001). However, additional studies are still necessary to elucidate the relevance of these mechanisms for biofilm resistance to biocides.

5.1.3.6 Role of persister cells

Recently, an alternate hypothesis was suggested to explain biofilm resistance to killing based mainly on studies of the efficacy of various antibiotics against biofilms. It is assumed that, similarly to multicellular organisms, damaged cells undergo a programmed cell death, while a small population of cells (persisters), which are defective in their suicide response, would survive the exposure to the antimicrobial agent in protected niches within the biofilm (Lewis, 2000, 2001). These persisters would benefit from defective biofilm organisms through nutrient provision and biocide quenching. Thus, biocide-induced damage triggers cell death and would eliminate defective cells within the biofilm population. When biocide treatment is discontinued, the persisters would start to multiply, producing a new biofilm population consisting mostly of biocide-sensitive cells and again only a minor fraction of new persisters. In contrast to induction processes and genetic mechanisms necessary for survival, persisters can react immediately and survive a sudden challenge by a biocide. Persisters are not mutants but arise at a considerably higher rate (10- to 10 000-fold) than mutants (Lewis, 2000). It was emphasized that dense populations of either stationary-phase cells or biofilms favors persister formation (Spoering and Lewis, 2001); this phenomenon was supposed to explain similar resistance of biofilm and stationary phase planktonic cells of *P. aeruginosa* to antibiotics, which were considerably more resistant than logarithmic planktonic cells. In a recent study, antibiotic-resistant variants of *P. aeruginosa* with an enhanced ability to form biofilms were shown to arise at high frequency in response to antibiotic treatment (Drenkard and Ausubel, 2002). It was speculated that some bacteria within a population undergo transient phenotypic changes to antibiotic-resistant variants, which are selected inside mature biofilms by antibiotic treatment; they switch back to the antibiotic-susceptible forms after antibiotic treatment.

These observations indicate that biofilm populations may always contain a sub-set of organisms which ensure survival of the species by the ability to adopt transiently a biocide-resistant phenotype.

5.1.4 Methods to study antimicrobial action on biofilms

In general, three methods can be applied to study the antimicrobial action of biocides against biofilms:

- Planktonic assays
- Dried surface assays (contaminated surfaces)
- Biofilm assays

5.1.4.1 *Planktonic assays*

The simplest way to investigate biofilm organisms is to remove and suspend them and to carry out further investigations with biofilm suspensions. However, highlighted by the background of observations presented earlier, suspended biofilm organisms are not biofilms any more. The difference originates from the disruption of the matrix, such that the extrapolation of results of such investigations to real biofilms was to be considered with great scepticism.

The only advantage of this approach is that test systems based on suspended bacteria are well standardized. The test organisms are cultivated in liquid nutrient media, subcultured in fresh media, harvested, washed, and resuspended in a buffer at a defined cell density. The test suspension is then brought into contact with the biocide. After a given contact time, a neutralising medium is used to inactivate the biocide, which is followed by determination of the number of surviving cells. This usually consists of determining the colony-forming units on nutrient-rich agar medium. The procedure represents a traditional and widely used method for determination of biocide efficacy. A series of recommendations, guidelines and “standards” for such purposes are available; these endeavour to standardise the procedures for tests of this type. However, results obtained from experiments with suspended biofilms have to be treated with great scepticism.

5.1.4.2 *Surface contamination experiments*

Surface contamination experiments are especially relevant in medicine and in the food industry, in terms of disinfection of instruments and work surfaces. They refer to microorganisms adhering to surfaces without physiological formation of further developed biofilms. However, they can be considered as microbial aggregates and differ from suspended organisms. For these applications, there are a series of standardised procedures at national levels, e.g. Germany (DGHM, 1991), France (AFNOR, 1988), Belgium (Reybrouck, 1990), The Netherlands (van Klingeren, 1978), and at the European level (CEN/TC 216, 1998). In contaminant-loading experiments, bacterial cells or spores are distributed on a surface, and then fixed to the surface by means of air- or vacuum-drying.

Carrier tests with dried cells on surfaces are not suitable for the evaluation of biocide efficacy towards biofilms. In general, microbial cells dried on carriers are less susceptible to biocides compared to planktonic cells; however, established biofilms grown on surfaces usually display enhanced resistance compared to organisms simply dried on carriers (e.g., Samrakandi et al., 1994; Ntsama-Essomba et al., 1997). A possible reason may be physiological changes which are associated with biofilm formation, and can result in enhanced resistance to biocides. These processes are not involved in carrier tests.

5.1.4.3 *Biofilm experiments*

There are currently no recommendations or guidelines concerning the standardized cultivation of biofilms; methods which give indications of practical efficacy are the most useful. Attempts have been made to unify procedures for determining the efficacy of biocides; however, the findings of the available literature are difficult or impossible to compare. In principle, this can be described under several headings with respect to the duration of the investigations:

- Cultivation of biofilms
- Biocide treatment
- Removal and homogenisation of the biofilm cells
- Determination of biocide efficacy

5.1.4.3.1 *Cultivation of biofilms.* The majority of biofilm studies are based on monocultures (Baldry, 1983; Christensen et al., 1990; Samrakandi et al., 1994; Johnston and Jones, 1995; Wood et al., 1996; Ntsama-Essomba et al., 1997; Blanchard et al., 1998; Härkönen et al., 1999; Lindsay and von Holy, 1999; Cochran et al., 2000;

Herruzo-Cabrera, 2000; Bredholt et al., 2001; Spoering and Lewis, 2001). The obvious advantage is that the organisms are well defined; the obvious disadvantage is their lack of representativity to environmental conditions. Defined mixed cultures are rarely employed (Alasri et al., 1992, Fatemi and Frank, 1999). The advantage of biocide studies with monocultures or defined mixed cultures lies in the better reproducibility of the experiment. In this way, variations in the experimental parameters can be chosen or kept constant.

Only in few publications is the action of biocides described for aqueous systems with undefined biofilms with mixed composition (Exner et al., 1987; Mathieu et al., 1990; Goroncy-Bermes and Gerresheim, 1996; Morin, 2000; Holtmann and Sell, 2001; Walker et al., 2001). The advantage of efficacy testing on natural biofilms lies in the greater relevance to the practical situation.

Vastly different methods are available for cultivation of biofilms. Basically, these can be described as either batch-mode or continuous mode methods. In batch studies, growth substrata in the form of coupons or glass slides are placed in e.g. Petri-dishes or other holders filled with medium. Under the action of undefined shear forces, investigations in so-called "beaker" reactors are conducted. A further test system which operates in batch mode and continues to become prominent is the miniaturized test system comprising microtitre plates, in which 96 wells enable the simulation of various experimental conditions simultaneously under static conditions (Geneveaux et al., 1996; O'Toole et al., 1999). In general, biocide tests in batch systems are simpler to run, have shorter durations, and are very simple to carry out. Therefore, they are well-suited for initial comparisons or screenings of different biocides, or different concentrations and contact times of a particular biocide.

Should the biocide require practice-orientated investigation and testing under defined conditions, continuous systems are best suited. The simplest continuous test system comprises a flow-through tube (preferably silicon which supports biofilm growth by release of softeners) which acts as a growth substratum (Exner et al., 1987; Mathieu et al., 1990). Equally uncomplicated is the exposure of test surfaces in continuous systems, such as the continuous-flow stirred-tank reactor (CSTR), which consists of a simple glass beaker through which medium is pumped continuously (Hamilton, 2002). The inoculation of biofilm reactors usually is considered as the beginning of the experiment. Often, the reactor is operated in batch mode for 24 hours to enable adaptation of the bacteria before the supply of medium is started. As a rule, the flow velocity is set such that biofilm cell growth is faster than suspended cell growth (Characklis, 1990). In this way, suspended bacteria are rinsed from the reactor.

After defined exposure times, the test substrata can be removed from the system and treated with biocide, or they remain in the system and are analysed at the end of the experiment to determine biocide efficacy. Other, new variations of the CSTR can be suitable as test systems. These include the Calgary Biofilm Device, which utilises shaken microtitre plates (Ceri et al., 1999; Ceri et al., 2001), the flow-cell (Stoodley et al., 2001), the artificial biofilm system (Harkonen et al., 1999), the colony biofilm system (Anderl et al., 2000) and the drip flow reactor (Xu et al., 1998).

More complex reactor systems for biofilm growth under defined conditions, i.e. controlled hydrodynamic conditions with the possibility of subsequent biocide treatment, include the RotoTorqueTM (rotating annular reactor) (Characklis, 1990), Robbins Device (McCoy et al., 1981), constant-depth film fermenters (Atkinson and Fowler, 1974; Peters and Wimpenny, 1988, 1989) or the radial flow (Fowler and McKay, 1980) and rotating disc reactors (Loeb et al., 1984). An introduction to these reactor systems is given by Gilbert and Allison (1993).

Rotating annular reactors are used in many water installations to analyse the mechanisms of biocide action. Investigations concerning the action of monochloramine and free chlorine on monoculture biofilms of *P. aeruginosa* were described by Chen et al. (1993a and b), Griebe et al. (1994) and Srinivasan et al. (1995). Christensen et al. (1990) employed a miniaturised rotating annular reactor to investigate the action of hydrogen peroxide in combination with Fe^{2+} on monoculture biofilms of various *Pseudomonas* species.

5.1.4.3.2 Biocide treatment. After establishment of the biofilm, biocide treatment is accomplished by exposure of test surfaces with attached biofilm to the biocide-containing solution. The biocide treatment is performed either by direct application of the biocide to the system in which the biofilm has become established, or separately, following removal of the growth substrata with the attached biofilm. In continuous systems, it is also possible to administer the biocide-containing solution to the entire system, and then rinse after the desired contact time. In this way, intermittent biocide treatment can be accomplished, as can investigations of the subsequent growth of the biofilm following a biocide treatment (recontamination). As in the case of the suspension tests, the procedure is followed by neutralisation of the biocide.

In the case of hydrogen peroxide, catalase is commonly chosen as the neutralising solution, since it cleaves the active agent into water and oxygen. If the biofilm is treated with chlorine, sodium thiosulphate is generally used as neutralising agent. Peroxidase stops the reaction in the case of peracetic acid, while silver compounds are inactivated with sodium thioglycolate or ascorbic acid. If more than one biocide is tested, the neutralisation solution contains a cocktail of the inhibitors, or a universal quenching agent (UQA), the latter of which generally includes peptone, Tween 80, sodium thiosulphate, catalase, and lecithin in deionised water (Lambert et al., 1998; Lambert and Ouderaa, 1999).

5.1.4.3.3 Removal and homogenisation of the biofilm cells. For the quantification of the effect of biocides, in most cases, the biofilm has to be removed from the test surface as it is difficult to quantify biofilms directly. The problem is the fact that bacteria which have been damaged previously by exposure to biocide are completely inactivated by a further, usually mechanical stress. As a consequence of careless removal and homogenisation, the action of a biocidal substance can be overestimated.

A widely used method for removal and simultaneous homogenisation of biofilms consists of treatment with ultrasound. As additional possibilities for removal of biofilm organisms, various methods are used to scrape the colonisation substrata. Depending on the composition of the test surface, rubber scrapers or razor blades can be suitable for complete removal of the biofilm. The removed bacteria can then be transferred to a defined volume of suspension medium. Subsequently, they are homogenised mechanically. Another method is removal of the biofilm with a sterile cotton swab, which is then transferred in its entirety to a test tube containing a suspension medium. After a defined homogenisation time (e.g., shaking), the swab is removed, leaving the suspended bacteria in the solution.

In test systems which are difficult to access, e.g., tubes, catheters or pipes, quartz sand is commonly used to remove biofilm. Approximately one-third of the respective device is filled with quartz sand of a suitable grain size (diameter of 0.5 to several millimetres), and the remainder is filled with suspension medium. By a particular agitation method, such as rotation of the test surface, the biofilm is abrasively removed and, together with the quartz sand, transferred to a sterile container. The quartz sand is removed by centrifugation, and the supernatant is shaken to homogenise the suspended bacteria.

5.1.4.3.4 Determination of biocide efficacy. As described previously, two basic aspects should be considered concerning the action of biocides:

1. the killing efficacy and,
2. the cleaning efficacy of the biocide.

The action of antimicrobial substances, from inhibition of metabolic activity to physical destruction of the microorganisms, is very complex and difficult to identify, in that microorganisms represent “multiple targets”. This means that, in general, introduction of an antimicrobial agent affects more than one cellular component. Therefore, it is difficult to differentiate between primary and secondary effects. Through judicious choice of different methods, it can be investigated with some selectivity whether the biocide inhibits metabolic activity, if particular enzymes are inactivated, and/or if destruction of the cell wall, respiratory apparatus, ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) are involved. By a combination of the following detection methods, conclusions can be made concerning the sites at which a biocide is active.

The number of surviving bacteria is generally measured by culture-based methods which are related to the surface colonising area following removal of the biofilm bacteria from the surface. Thereby, all bacteria which are still capable of multiplication under particular conditions (medium composition, incubation temperature/time) are considered. These methods commonly do not reflect the real situation in a biofilm because in this case, many organisms show activity but cannot be cultivated (viable but non culturable, “VBNC”, Kell et al., 1998). Thus, the numbers of viable organisms measured by traditional cultivation-based methods are usually grossly underestimated. Certainly, a considerable advantage of these methods is that they have been employed for a long time and therefore, even when the measured colony counts do not reflect the actual cell number, they are process-relevant and give some practical measure of the microbial burden in a system.

A somewhat modified cultivation-based method, which allows the metabolic activity of surface-attached bacteria to be recognised, consists of coating test coupons in Petri dishes with a layer of agar, followed by overnight incubation. The agar is then removed from the surface and incubated further with the contact-side facing up. The metabolically active bacteria form colonies which can be counted (Bredholt et al., 1999).

A further possibility to determine the active cells in biofilms is the application of fluorescent dyes. The advantage of these methods is the short incubation time. Within a few hours, the required information is obtained, whereas cultivation-based methods can require days or even weeks. The determination of cell showing respiratory activity is performed with e.g., the tetrazolium salt 5-cyano-2,3-ditolyl tetrazolium chloride (CTC, Schaule et al., 1993). Another microscope-based detection method for active and inactive bacteria involves staining with the fluorochromes contained in the Live/Dead *BacLight* Bacterial Viability Kit. This comprises two fluorochromes, both of which bind to DNA. The differentiation between “living” and “dead” cells is based on membrane integrity. Also, the ATP content can be measured with the surface monitoring kit 1243-114; this is a measure of the metabolic activity of biocide-treated bacteria (Bredholt et al., 1999).

It is also possible to measure the vitality of biofilm bacteria through measurement of the transmembrane potential with Rhodamine 123 or DiBAC₄(3) (Comas and Vives-Rego., 1997; Lisle et al. 1999).

The ability of biocide-treated biofilms to utilise nutritional substrates can be determined by a “direct viable count” (DVC), which is measured by the effect of nalidixic acid (Lisle et al., 1999). A further method described by Holtmann and Sell (2001) for indirectly measuring the metabolic activity of biocide-treated bacteria is the determination of redox potential in the biofilm with microelectrodes. It can be shown that the increase of redox potential is correlated with a reduction in the colony count and the dehydrogenase activity. Microelectrodes are proposed as a way of monitoring the success of biocide treatments.

Less commonly, an additional measurement is used to determine the removal of biofilm bacteria. In this way, the surface-associated total cell count (i.e., active and inactive cells) can be determined microscopically following staining of the cells with fluorescent dyes. Suitable dyes for determination of total cell counts include 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), Acridine Orange (AO), Syto 9, and Sytox Green. These bind to DNA or, in some cases, also to RNA. Depending on biofilm thickness, the biofilm cells on a surface can be quantitated directly or after suspension and filtration onto a black (non-fluorescing) membrane filter. A further possibility for detection of surface-associated, stained bacteria consists in surface coverage by means of image analysis (Wirtanen and Mattila-Sandholm, 1996).

An assessment of the the biofilm thickness and internal structure can be performed by observation of the surface with a confocal laser scanning microscope (CLSM). With CLSM, any change in the morphology of the biofilm resulting from biocide treatment can be detected. Often, an additional analysis of the surface-associated biofilm before and after biocide treatment is made using scanning electron microscopy (SEM).

5.1.5 Selected biocides

For information on the general activities and mechanisms of action of biocides, the reader is referred to recent reviews on this subject (McDonnell and Russell, 1999; Donlan and Costerton, 2002) and to other chapters of this book. We have not attempted to present an exhaustive overview on the entire range of biocides and their efficacy against biofilms. Instead, some examples have been selected.

5.1.5.1 Chlorine-containing compounds [II, 21.2]

Because of its wide use in industrial water systems and clinical settings, much information exists about the effect of free available chlorine on biofilms grown in different environments. Other chlorine-based compounds which have been used in the control of biofilms are chlorine dioxide and chloramines. The term “chlorine” is broadly used to signify “active chlorine compounds” in aqueous solution, consisting of a mixture of Cl_2 , OCl^- , HOCl and other active chlorine compounds (Dychdala, 1991). Chlorine is one of the most commonly used chemicals for the control of biofouling; however, side reactions with organic and inorganic compounds in the bulk water or with substratum material may result in substantial chlorine consumption and may render the biocide less effective; pH and temperature also have a strong influence on chlorine efficacy (Dychdala, 1991). Thus, water quality, choice of on equipment surfaces, but also hydrodynamic conditions are important factors that determine the efficacy of chlorine in water systems. An extensive process analysis of biofouling control with chlorine was given by Characklis (1990).

In water at pH values between 4 and 7, chlorine predominantly exists as hypochlorous acid (HOCl); it is in equilibrium with the hypochlorite ion (OCl^-), which predominates above pH 9. Hypochlorous acid is the rapidly acting component, and is mainly responsible for the microbicidal activity of chlorine. Thus it is suggested that low pH values enhance the biocidal activity, whereas high pH values (> 7) favour hypochlorite ion-mediated disruption and detachment of mature biofilms (Characklis, 1990). In this respect, data from the literature for chlorine efficacy on biofilms are difficult to compare because either different pH values were used or pH values are not mentioned. Moreover, most studies focus on the killing of biofilm organisms, while a limited number of studies also include the aspect of biofilm detachment from surfaces (Table 1).

In general, field observations and laboratory studies indicate that biofilms seem to be recalcitrant to the killing and disrupting activity of chlorine in concentrations which are relevant in practice, whereas planktonic cells are more easily inactivated by the biocide. Biofilm bacteria have been shown to persist in the presence of relatively high chlorine concentrations. For example, maintenance of a 1.0 mg/L free chlorine residual was insufficient to control coliform growth in drinking water distribution biofilms (LeChevallier et al., 1987). Experiments in a pilot plant showed that 4 mg/L free chlorine was not sufficient to kill bacteria in an established biofilm on iron surfaces (LeChevallier et al., 1990). After an exposure of biofilms on PVC pipe surfaces to free chlorine concentrations of 10 to 15 mg/L for seven days, biofilm bacteria (pseudomonads and mycobacteria) were shown by scanning electron microscopy (SEM) to survive in the form of EPS-enclosed microcolonies attached to the PVC walls (Vess et al., 1993); a few days after exposure to the disinfectant, the bacteria could be recovered again from the water phase.

Table 1 Efficacy of chlorine (sodium hypochlorite) against biofilms (examples). n.d., not determined

System	Material	Organisms	Age of biofilm	Product	Contact time	Concentration	Reduction in viable cell counts	Detachment of biofilms	References
Pipe system	PVC	pseudomonads, mycobacteria	8 w	sodium hypochlorite	7 d	10–15 ppm free chlorine	incomplete	incomplete	Vess et al., 1993
Tube system	silicone	tap water biofilm	50 d	chlorine	24 h	0.3 mg/L Cl ₂ , 10 mg/L Cl ₂	none, 4 log units	none	Exner et al., 1987
Tube system	Tygon	<i>B. subtilis</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	5 d	sodium hypochlorite	60 min	0.5% sodium hypochlorite	completely (> 6 log units)	effectively (1% sodium hypochlorite)	Alasri et al., 1992
Batch system	stainless steel	<i>Pseudomonas</i> sp., <i>Listeria monocytogenes</i>	2 d	hypochlorite	1 min	160 mg/L	> 8 log units	n.d.	Fatemi and Frank, 1999
Tube system	Tygon	<i>B. subtilis</i> , <i>B. megaterium</i>	> 3 d	sodium hypochlorite	5 min, 180 min	735 mg/L chlorine 1439 mg/L chlorine	< 1 log unit 2.5 – 2.8 log units	n.d.	Samrakandi et al., 1994
Tube system	PVC	<i>E. coli</i>	5 d	sodium hypochlorite	5 min	195 mg/L chlorine	> 5 log units	n.d.	Ntsama-Essomba et al., 1997
Annular reactor	stainless steel	<i>P. aeruginosa</i> , <i>K. pneumoniae</i>	7–9 d	sodium hypochlorite	60 min	15 mg/L, pH 6.4 15 mg/L, pH 10.9	41% reduction 99.6% reduction	47% removal 65% removal	Chen and Stewart, 2000
Flow system	stainless steel	<i>P. aeruginosa</i> , <i>K. pneumoniae</i>	6 d	sodium hypochlorite (pH 11)	60 min	1000 mg/L	0.85 log units	partially	Stewart et al., 2001

Exner et al. (1987) reported that 10 mg/L of free chlorine reduced the colony count of drinking water biofilms on silicone tube surfaces by approximately four log units after a contact time of 24 h; however, no complete inactivation was observed. SEM examination of the silicone surfaces demonstrated that no biofilm removal had occurred. A shorter contact time of 60 min or treatment with a lower chlorine concentration (0.3 mg/L) for 24 h had no inactivating effect on the biofilm bacteria.

Meiller et al. (2001) treated tubing sections from a dental unit waterline with bleach at the working concentration recommended by the manufacturer (0.52% sodium hypochlorite). After 18 h exposure, cultures were negative for biofilms, indicating antimicrobial efficacy of the product. However, SEM analysis revealed that the biocide failed to totally disrupt or remove the biofilms from the tube surfaces.

A minimum concentration of 0.5% sodium hypochlorite at a contact time of 60 min was necessary to achieve a complete inactivation of a mixed-culture biofilm (*E. coli*, *P. aeruginosa*, *B. subtilis*) on Tygon tube surfaces (Alasri et al., 1992); the same result was obtained with 2% sodium hypochlorite within 10 min. SEM analysis showed that application of 1% sodium hypochlorite for 60 min resulted in an effective detachment of the biofilms.

Chlorine has been reported to have limited efficacy against spore-containing biofilms (Samrakandi et al., 1994). Less than one log unit reduction of colony counts was observed when pure-culture biofilms containing spores of either *B. subtilis* or *B. megaterium* were treated with chlorine (735 mg/L, *B. subtilis*; *B. megaterium*, 1439 mg/L) for 5 min; even after a contact time of 180 min, colony counts decreased by less than 3 log units.

Flint et al. (1999) reported that for planktonic cells of *Streptococcus thermophilus*, no viable cells survived the exposure to 20 mg/L of sodium hypochlorite for 30 min (pH 6.8–7.0), whereas viability of this organism grown as a biofilm on stainless steel was still detected after treatment with up to 1 000 mg/L of sodium hypochlorite.

One-day-old biofilms of *P. aeruginosa* on silicone disks were inactivated to below the detection limit (> 5 log reduction of viable counts) after 10 min exposure to 0.1% sodium hypochlorite, or after 30 min exposure to 0.01% sodium hypochlorite, while the same degree of inactivation for planktonic bacteria was achieved in less than 1 min (Takeo et al., 1994).

Ntsama-Essomba et al. (1997) reported a minimal bactericidal concentration (decrease of colony counts by 5 log units) of 195 mg/L available chlorine when 5-day-old *E. coli* biofilms on PVC surfaces were treated for 5 min with a sodium hypochlorite-containing product; the minimal bactericidal concentration towards planktonic cells was five times lower. The age of biofilms influenced chlorine activity; the minimal bactericidal concentration towards 10-day-old biofilms was 10 times higher than for 5-day-old biofilms (Ntsama-Essomba et al., 1997). A similar observation was reported by LeChevallier et al. (1988a). Reduction of viable counts of 2-day-old *K. pneumoniae* biofilms was more than two log units higher than for 7-day-old biofilms, when the biofilms were exposed for 10 min with 1 mg/L of free chlorine (pH 7.0). No age-related increase in biofilm resistance was observed when the biofilms were treated with 5 mg/L of monochloramine under the same conditions (LeChevallier et al., 1988a).

Dual-species biofilms of *P. aeruginosa* and *K. aerogenes* grown for 6 days were highly resistant to killing by alkaline hypochlorite (Stewart et al. 2001). Treatment of biofilms with 1000 mg/L of the biocide for 1 h resulted in a 0.85 log reduction of the viable cell numbers, while similar treatment of planktonic cells led to a greater than 6 log reduction of the viable cell numbers. The average removal (65% of total cells) effected by hypochlorite dissolved in carbonate buffer (pH 11) was the same as the buffer without added hypochlorite. A similar observation was reported for biofilms grown in a continuous flow annular reactor system (Chen and Stewart, 2000). The efficacy of chlorine treatment at neutral pH was attributed to hypochlorous acid, while the efficacy of alkaline hypochlorite treatment was supposed to be due to the high pH value, and not the halogen (Chen and Stewart, 2000).

Using microelectrodes for monitoring biocide penetration into biofilms, a number of studies showed that transport of chlorine into biofilms was clearly retarded (de Beer et al., 1994b; Chen and Stewart, 1996; Xu et al., 1996; Stewart et al., 2001). This effect seems to be due to its interaction with cells and/or EPS in the biofilms, resulting in consumption and neutralization of chlorine. If chlorine has a higher reaction rate with biofilm components, then it will penetrate more slowly into the deeper layers of a biofilm. The chemical reaction between the extracellular polysaccharide alginate as one of the major EPS components in biofilms of *P. aeruginosa* and chlorine has been demonstrated (Wingender et al., 1999); *O*-acetyl groups in this alginate were mainly responsible for the reaction with chlorine, as removal of acetyl groups abolished the reactivity of the polysaccharide.

The chlorine-based biocides monochloramine and chlorosulfamates were reported to penetrate better into biofilms than free chlorine, because these compounds seem to have lower capacity for reaction with biofilm constituents (Griebe et al., 1994; LeChevallier et al., 1998b; Stewart et al., 2001). Although both compounds are weaker biocides than chlorine, they were shown to be similarly effective when tested against biofilms; however, their killing action was only very limited as was also observed for chlorine. The efficacy of chlorine and monochloramine was compared towards *P. aeruginosa* biofilms grown in an annular biofilm reactor (Griebe et al., 1994); a dose of 4 mg/L of monochloramine was found to be more effective for biofilm inactivation than a dose of 10.8 mg/L of free chlorine. Binary population biofilms of *P. aeruginosa* and *K. pneumoniae* grown for

7–9 days in a continuous flow annular reactor were killed more effectively by monochloramine than by free chlorine when treated with equivalent concentrations for 1 h at neutral pH (Chen and Stewart, 2000); however, the amount of biofilm removed by free chlorine and monochloramine was not statistically different. Rao et al. (1998) reported similar biocidal activity of monochloramine and free chlorine towards 2-day-old biofilms of sea-water bacteria at similar doses of up to 3 mg/L and contact times of up to 60 min. The biofilm thickness was reduced by 90% in the case of both biocides; cell detachment was 94% and 100% for monochloramine and free chlorine, respectively (Rao et al., 1998). Yu et al. (1993) observed comparable disinfection efficiencies of 0.25 mg/L free chlorine (at pH 7.2) and 1 mg/L monochloramine (at pH 9.0) towards young *K. pneumoniae* biofilms; however, monochloramine was more effective in removing attached bacteria than free chlorine. LeChevallier et al. (1990) reported that biofilms in a model water distribution system were successfully controlled using monochloramine levels ranging from 2 to 4 mg/L; free chlorine residuals from 3 to 4 mg/L were ineffective in reducing the viability of biofilm bacteria grown on iron pipes. Based on these observations, it can be suggested that monochloramine is more effective against certain biofilms since it reacts more slowly in side reactions and thus is able to penetrate further into biofilms (LeChevallier et al., 1990).

Relatively few studies have included the effect of chlorine dioxide on biofilms. Characklis (1990) mentions that chlorine dioxide has been successfully used to control biofouling in several industrial environments. Walker and Morales (1997) studied the effect of chlorine dioxide on a mixed population of drinking water bacteria in a continuous culture model which was developed to simulate an industrial water system. The addition of 1 mg/L chlorine dioxide for approximately 18 h was sufficient to reduce the viable counts of the planktonic population by 99.9%, whereas 1.5 mg/L chlorine dioxide was required to achieve a similar reduction in the biofilms, suggesting an enhanced resistance of biofilm bacteria to the biocide. There are indications that continuous disinfection of drinking water using chlorine dioxide provides a certain control of biofilm formation. In a French drinking water distribution system, the presence of chlorine dioxide allowed a limited surface colonization, while in regions where chlorine dioxide was below the detection limit, an increase in biofilm formation occurred (Servais et al., 1995).

5.1.5.2 Ozone

Ozone has been used for the treatment of drinking water and wastewater, and has been proposed as a promising biocide in industrial cooling water systems due to its rapid action against planktonic microorganisms (Wickramanayake, 1991; Viera et al., 1999a). However, only few reports exist on the action of ozone against biofilms. In a study on *P. fluorescens*, the efficacy of ozone against sessile bacteria was lower than that found for planktonic cells (Viera et al., 1999a, b). Treatment of single-species biofilms of *P. fluorescens* with ozone (0.18–0.50 mg/L) for maximally 60 min resulted in a limited decrease in viable cell counts of only up to three orders of magnitude (Viera et al., 1999a); scanning electron microscopy revealed that the effect of ozone also included detachment of the cells of sessile bacteria from the steel surfaces. In the same study, viable counts in ozone-treated mixed-species biofilms of sulfate-reducing bacteria decreased by one to three orders of magnitude. The penetration of ozone into the *P. fluorescens* biofilms appeared to be a function of simultaneous diffusion in the biofilm matrix and reaction of the biocide with biofilm components such as cell mass, lysis products or EPS (Viera et al., 1999b). However, EPS may not always be reactive with ozone; thus, exopolysaccharides of *K. aerogenes* did not protect the bacteria from the action of ozone, since the sensitivity of encapsulated cells was not significantly different from that of an isogenic capsule deficient strain (Falla and Block, 1987).

5.1.5.3 Peroxygens

5.1.5.3.1 Hydrogen peroxide [II, 21.1.1]. Hydrogen peroxide (H_2O_2) is being introduced more frequently into practice as a biocide due to its desirable environmental properties compared with the more conventional application of chlorine compounds. The breakdown products from the decomposition of hydrogen peroxide are water and oxygen, enabling simple waste disposal.

H_2O_2 has proven to be bactericidal against numerous Gram-negative and Gram-positive bacteria; against many viruses, hydrogen peroxide has a delayed action (overview in: Block, 1991b). In relatively high concentrations (10 to 25% w/v), hydrogen peroxide also displays sporicidal properties. It is assumed that the bactericidal activity of hydrogen peroxide functions principally through the production of hydroxyl radicals. The generation of hydroxyl radicals can take place through interaction with metal ions, e.g. iron (Fenton reaction) (Block, 1991). The highly-reactive hydroxyl radicals can attack membrane lipids, proteins, DNA, RNA and numerous other cellular macromolecules. Further susceptible positions are represented by sulfhydryl groups and double bonds in proteins and lipids, which are oxidized by hydrogen peroxide.

Hydrogen peroxide alone and in practice-relevant concentrations shows only weak inactivation efficacy against microorganisms in comparison with, e.g. chlorine; however, it does cause a dissolution and partial removal of biofilms (Exner et al., 1987; Christensen et al., 1990). It seems that intermittent application of

hydrogen peroxide leads to biofilms that are difficult to remove (Christensen et al., 1990). In dried surface assays with various bacterial species, hydrogen peroxide always demonstrates a good bacteriostatic effect against attached bacteria, but poor bactericidal activity (Baldry, 1983). For comparison, planktonic cells are also tested. The disinfection rate for planktonic cells is significantly higher compared with that of 20 h or older biofilms. The resistance of biofilm bacteria to hydrogen peroxide increases with the age of the biofilm (Cochran et al., 2000). An overview of the published work is shown in Table 2.

Other commercially available hydrogen peroxide based formulations also contain solubilised colloidal silver, whereby a synergistic effect is expected; an increase in the disinfection efficacy is claimed (Pedahzur, 1995). Various results have been published concerning the action of hydrogen peroxide and silver combinations. These mostly apply to laboratory investigations of suspended cells and pure cultures, where biofilms are not taken into consideration. It has been established that when silver ions are added to hydrogen peroxide, the combined action can be different from the individual components. In laboratory investigations, it has been shown that the combination of silver and hydrogen peroxide in a mass ratio of 1:1000 (in the range of 5 to 30 µg/L silver and 5 to 30 mg/L hydrogen peroxide) results in a higher inactivation performance in comparison to either component alone. Here, a weak synergistic effect has been described, such that the combined efficacy is higher than the sum of the individual inactivations through silver and hydrogen peroxide, respectively (Pedahzur et al., 1995). A similar synergistic effect has been observed in another investigation involving suspended cells (Potapchenko et al., 1996). A comparison of commercial hydrogen peroxide solution and a silver-containing hydrogen peroxide preparation (49.5% v/v hydrogen peroxide, 905 mg/L silver) suggests that there is no significant difference in the inactivation efficiencies against bacterial suspensions (Schiffmann, 1994).

5.1.5.3.2 Peracetic acid [II, 21.1.3]. There are numerous studies concerning the action of peracetic acid which are again concerned with investigations of suspended bacteria (Flemming, 1984; Block, 1991b). Peracetic acid-containing products are commonly employed in the dairy and beverage industries due to their broad-spectrum microbial action and good ecological properties (e.g. degradability) (Orth, 1998). Peracetic acid is used in clinical environments for decontamination of surgical endoscopes and associated equipment as an alternative to the typically used glutaraldehyde (Ayliffe, 2000).

A series of publications deals with the action of peracetic acid on wastewater organisms (Mathieu et al., 1990; Sánchez-Ruiz et al., 1995; Rajala-Mustonen et al., 1997; Lazarowa et al., 1998; Liberti and Notarnicola, 1999). Here, the emphasis is on the potential practical applications of peracetic acid for disinfection, i.e. for inactivation of disease-causing organisms in the original sense. These results are also of interest from the point of view of action of peracetic acid on biofilms; it is assumed in this case that pathogenic organisms are present not as individual bacterial cells or virus particles, but rather as aggregates of faecal origin. Peracetic acid should also be active against disease-causing organisms in aggregated or immobilised form, similar to the case of biofilms.

The few publications which discuss the action of peracetic acid against drinking water or wastewater biofilms suggest that practice-relevant concentrations of peracetic acid cause a relatively fast and effective inactivation of biofilm bacteria, but result in no or minimal removal of the biofilm under these conditions. This is considered to be a disadvantage for at least the drinking water area, where residual biofilm material on surfaces can promote recontamination of the water (Exner et al., 1987; Morin, 2000). In this context, Mathieu et al. (1990) reported that the discontinuous application of peracetic acid led to an incomplete disinfection and dissolution of biofilms, but nevertheless can significantly limit the extent of biofilm development.

Alasri et al. (1992a) conclude that the combination of peracetic acid and hydrogen peroxide exerts a complementary effect on biofilms; peracetic acid has a clear microbicidal effect given that the hydrogen peroxide has a successful effect on biofilm detachment. The detached bacteria were significantly more sensitive to peracetic acid than the same bacteria in biofilms on the steel surface. It was therefore concluded that the complex structure of the biofilm and its attachment to the surface are responsible for the protective effect against peracetic acid, rather than a physiological adaptation of the biofilm bacteria with increasing resistance against peracetic acid (Johnston and Jones, 1995).

An overview of investigations concerning the effects of peracetic acid against biofilms is shown in Table 3.

5.1.5.4 Silver compounds

Silver and its compounds, including silver sulfadiazine and silver nitrate, have long been used as antimicrobial agents (Russell and Hugo, 1994). Some studies indicate that the incorporation of silver into paints and coatings for use in industrial water systems and in medical devices seems to be suitable to impair adhesion and delay biofilm formation, while in other studies no protective effect of silver has been observed.

Under the aspect of its application in water systems, a silver-containing paint was shown to retard surface colonization and growth of heterotrophic bacteria, including the pathogen *Legionella pneumophila*, for up to 14 days compared to control glass surfaces without the silver paint (Rogers et al., 1995). After prolonged incubation, the silver paint lost its protective effect against biofilm development, (Rogers et al., 1995) probably due to

Table 2 Efficacy of hydrogen peroxide (H₂O₂) against biofilms. n.d., not determined

System	Material	Organisms	Age of biofilm	Product	Max. contact time	Concentration	Killing	Detachment	References
Tube system	silicone	Tap water biofilm	50 d	H ₂ O ₂	60 min	1.5 a. 2% H ₂ O ₂	more than 5 log-units	partly	Exner et al., 1987
Tube system	Tygon-tube	<i>B. subtilis</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	5 d	H ₂ O ₂ (30/35% Produits chimiques du Ciron)	60 min	4% H ₂ O ₂	completely (more than 6 log-units)	no	Alasri et al., 1992a
Hollow fibre	No information	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	1 h	H ₂ O ₂ (30/35% Produits chimiques du Ciron) with PAA (Interox Chemicals)	4 d	97.65 mg/L H ₂ O ₂ in combination with 0.75 mg/L PAA	completely (more than 6 log-units after 5 h contact time)	n.d.	Alasri et al., 1992b
Batch-reactor with pieces of pipes	stainless steel	Isolates from disinfectant dosage device	48 h	H ₂ O ₂	60 min	1% H ₂ O ₂	0.88 to 3.77 log units depending on the species	n.d.	Goroney-Bernes a. Gerresheim, 1996
Micro-annular reactor (MAR)	no information	<i>Pseudomonas</i> sp. NCMB 2021	30 h	H ₂ O ₂	6 h	150 mM H ₂ O ₂	n.d. (online monitoring with turbidity measurements)	84%	Christensen et al., 1990
Dried surface assay	steel	<i>P. atlantica</i> ATCC 19262 Spores of <i>B. subtilis</i> ATCC 15441	24 h (dried cells)	H ₂ O ₂ (35% Interox Chemicals Ltd)	6 h 24 h	1.5 mM H ₂ O ₂ and 0.1 µM Fe ²⁺ 0.88 mol/L, pH 4–8	72% 1 of the 14 tested carriers retains viable spores	n.d.	Baldry, 1983
Fed batch-reactor with alginate beads	alginate	<i>P. aeruginosa</i> PAO1	24 h	H ₂ O ₂	1 h	600 mg/L H ₂ O ₂	Disinfection coeff. 8.8 10 ⁵ L/mg min	n.d.	Cochran et al., 2000
Batch-reactor with cellulose carriers (3 M)	cellulose	<i>B. subtilis</i> (spores)	48 h	1 h H ₂ O ₂	600 mg/L H ₂ O ₂ 20 min	Disinfection coeff. 9.7 10 ⁵ L/mg min 11% H ₂ O ₂	less than 6 log units	n.d.	Herruzo-Cabrera, 2000

Batch-reactor with coupons in poloxamer-gel	steel	<i>M. luteus</i> , <i>S. epidermidis</i> , <i>S. typhimurium</i> , <i>S. worthington</i> , <i>E. coli</i> , <i>L. monocytogenes</i> , <i>L. innocua</i> , <i>P. aeruginosa</i> , <i>P. fluorescens</i> , <i>B. subtilis</i>	5 h	H ₂ O ₂ /PAA	5 min	0.5% product	0.2 to 2 log-units	n.d.	Härkönen et al. 1999
Dried surface assay	Steel and silicone	<i>B. subtilis</i> subsp. <i>globigii</i> (spores)	dried cells	20% H ₂ O ₂ and 4% PAA (Renalin)	11 h	1:5 dilution of the product	up to 5 log units	n.d.	Sagripanti and Bonifacino, 1999
Dried surface assay	Steel and silicone	<i>P. aeruginosa</i> ATCC 15442	dried cells	30% (Aldrich chemical Co.)	30 min	10%	2 log units (steel) and 4.4 log units (silicone)	n.d.	Sagripanti and Bonifacino, 1999
				20% H ₂ O ₂ and 4% PAA (Renalin)		1:5 dilution of the product	4.5 log units (steel) and 6.4 log units (silicone)		
Batch-reactor (cassette) with 16 discs	Tylon and trylon with incorporation of Cu phthalo-cyanine (PC) or CoPC	<i>P. aeruginosa</i> PaWH	24 h	H ₂ O ₂	30 min	3 mg/L H ₂ O ₂	1.5 log units (control) 1.5 log units (CuPC) 2 log units (CoPC)	Biofilm on catalyst containing discs easier to remove	Wood et al. 1996

Table 3 Efficacy of peracetic acid (PAA) against biofilms. n.d., not determined

System	Material	Organisms	Age of biofilm	Product	Max. contact time	Concentration	Killing	Detachment	References
Tube system	Silicone	Tapwater-biofilm	50 d	Stabilized PAA, Tenside	60 min	0.5 u. 1% product	more than 6 to 7 log-units	no	Exner et al. 1987
Tube system	Tygon	Sewage-biofilm	4 d	PAA with H ₂ O ₂	30 min	2.5 mg PAA/L 30 mg PAA/L	up to 97% n.d.	no ca. 40%	Mathieu et al. 1990
Activated carbon filter	Activated carbon	Mixed culture	45 d	PAA	10 min	100 mg PAA/L	not determined at the surface	partly	Morin, 2000
Tube system	Polyurethane-tubes	Tapwater-biofilm	14 d	PAA with H ₂ O ₂ a. acetic acid (Spor-Klenz)	16 h	no information (solution ready for use)	100%	92.6%	Walker et al. 2001
Tube system	Tygon-tube	<i>B. subtilis</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	5 d	PAA (Interox Chemicals)	60 min	0.5% PAA	completely (more than 6 log-units)	no	Alasri et al. 1992a
Batch-reactor with coupons	steel	<i>Pseudomonas sp.</i> , <i>Listeria monocytogenes</i>	2 d	PAA (Oxonia Active)	1 min	160 mg PAA/L	more than 8 log-units	n.d.	Fatemi a. Frank, 1999
Batch-reactor with coupons	steel	<i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>S. aureus</i>	4 h 20 h	PAA (Lever Industrial Ltd)	5 min 5 min	500 mg/L PAA 50 mg/L PAA 50 mg/L PAA	completely not completely (2-3 log-units) almost completely	n.d. n.d. n.b.	Johnston a. Jones, 1995
Batch-reactor with coupons	steel	<i>P. aeruginosa</i>	24 h	PAA (Proxitane, Solvay Interox R&D)	5 min	150 mg/L PAA	3 log-units	n.d.	Blanchard et al. 1998
Modified Robbins device	steel					30 mg/L PAA	3 log-units	n.d.	
Tube system	Tygon-tube	<i>B. subtilis</i> , <i>B. megaterium</i>	> 3 d	PAA (32%, Aldrich)	5 min 180 min	1222 mg/L PAA 1222 mg/L PAA	< 1 log-units max. 2.9 log-units	n.d. n.d.	Samrakandi et al. 1994
Tube system	PVC-tube	<i>E. coli</i>	5 d	PAA (2.5% with 18% H ₂ O ₂ , Bactipal)	5 min	125 mg/L PAA and 900 mg/L H ₂ O ₂	more than 5 log-units	n.d.	Ntsama-Essomba et al. 1997
Batch-reactor with coupons	steel	<i>B. subtilis</i>	34 h	PAA/H ₂ O ₂	1 min	170 mg/L product	1 log-unit	n.d. n.d.	Lindsay a. von Holy, 1999
Batch-reactor with coupons in poloxamer-gel	steel	<i>P. fluorescens</i> <i>M. luteus</i> , <i>S. epidermidis</i> , <i>S. typhimurium</i> , <i>S. worthington</i> , <i>E. coli</i> , <i>L. monocytogenes</i> , <i>L. innocua</i> , <i>P. aeruginosa</i> , <i>P. fluorescens</i> , <i>B. subtilis</i>	24 h 5 h	PAA/H ₂ O ₂	1 min 5 min	170 mg/L product 0.5% product	2.5 log-units 0.2 to 2 log-units	n.d.	Härkönen et al. 1999
Batch-reactor with coupons	steel	<i>P. fragi</i> , <i>L. monocytogenes</i> , <i>B. thuringiensis</i>	6 d	PAA (Oxonia Aktiv) + alkaline cleaner	5 min	1% product	Decrease to approx. 10 ³ cfu/cm ²	< 90%	Bredholt et al. 2001

Batch-reactor with papermill conditions	no information (reactor wall)	Isolate from a paper machine	48 h	PAA (5% with 23% H ₂ O ₂ , Proxitane 5, Solvay Interox)	point dosing	125 mg/L PAA	Increasing redox potential (indicates inactivation of several log-units)	n.d.	Holtmann a. Sell, 2001
Calgary biofilm device	polystyrol	<i>P. aeruginosa</i>	18 h	PAA	6 h	150 mg/L 300 mg/L	no killing more than 4 log-units	n.d.	Spoering a. Lewis, 2001
(Microtiter plates with pins)						400 mg/L	more than 6 log-units		

the resistance of silver from the surface of the paint. Silver-impregnated carbon filters are produced for point-of-use water treatment units. Based on literature data, it was concluded that, the average, no significant differences existed between silver-containing and nonsilver carbon units (Bell Jr., 1991). Silver-impregnated carbon units may become colonized with heterotrophic bacteria, which are released into the filter effluent, so that in these units silver does not usually confer a significant long-term effect on the treated water (Bell Jr., 1991). Silver coating has been suggested to prevent microbial growth on ion exchangers. Such coatings could suppress biofilm formation only for a period of a few weeks until a microbial population developed which could tolerate silver in much higher concentrations as actually present in the silver coated ion exchanger material (Flemming, 1987).

Adhesion and biofilm formation on catheters and implant materials by opportunistic microorganisms is a significant cause of infections patient in hospital. An approach to protect medical devices from microbial adhesion and biofilm formation has been to treat the surfaces with silver-containing coatings, to use silver-impregnated polymers or to produce silver ions electrically. The adhesion of Gram-positive and Gram-negative bacteria, and of the yeast *Candida albicans* was shown to be reduced on silver-coated catheters compared to non-coated controls (Gabriel et al., 1996; Gatter et al., 1998; Ahearn et al., 2000). Scanning electron microscopy (SEM) revealed that adhered cells of *P. aeruginosa* on hydrogel/silver-coated catheters after appeared damaged more often than on untreated catheters 18 hours (Gabriel et al., 1996). The biofilm development of *P. aeruginosa* monitored over 5 days was inhibited on catheters coated with silver at concentrations of 100 µg/mL and higher (Gu et al., 2001). In this study, the best protection against biofilm formation was achieved using a combination of 100 µg/mL silver and a lectin coating; this effect was probably due to the antimicrobial activity of silver ions and adhesion blockage from the lectins (Gu et al., 2001).

Electrically generated silver ions prevented the migration of *Staphylococcus epidermidis* through a silicone catheter over a 40-day period; this silver ion toporetic catheter had a broad spectrum inhibitory activity against bacteria (*S. epidermidis*, *S. aureus*, *Acinetobacter baumannii*) and a yeast (*C. albicans*) (Raad et al., 1996).

Other authors reported no microbicidal effect of silver. The survival of three bacterial species and *C. albicans* on a silver-impregnated polymer was not found to be influenced by the silver incorporation (Kampf et al., 1998). Using confocal laser scanning microscopy, Cook et al. (2000) observed that a silver-coated prosthetic heart valve sewing cuff was colonized by a higher number of bacteria (*S. epidermidis*) than an uncoated cuff. McLean et al. (1993) reported that only a combination of silver and copper in multilayer surface films on catheter materials provided enhanced antimicrobial activity compared to uncoated or only silver-coated surfaces.

These observations show that depending on silver ion availability at surfaces of medical devices, adhesion and biofilm growth may be prevented or at least delayed, which may be beneficial, for example, for catheterized patients. A recently published meta-analysis of the effectiveness of silver-coated urinary tract catheters indicated a significant benefit in patients receiving silver-coated catheters; silver alloy catheters were significantly more effective in preventing urinary tract infections than were silver oxide catheters (Saint et al., 1998).

Silver and copper ions act synergistically in the killing of *Legionella* bacteria, which are known to multiply in biofilms in hot water distribution systems. Copper-silver ionization has been used successfully to control *Legionella* spp. in many US hospital hot water systems after 5 to 11 years of operation; however, high pH values and elevated chloride concentrations have negative effects on the biocidal efficacy of copper and silver, respectively, in water systems (Lin et al., 2002).

The effect of silver ion addition to established biofilms was investigated in several studies. Spratt et al. (2001) reported that treatment with 5 mg/L colloidal silver for 1 hour was ineffective in the killing of 48-hour membrane-grown single-species biofilms of root canal isolates. The colony counts in biofilms of *S. aureus* after exposure for 24 hours to silver sulfadiazine or to silver nitrate in rabbit plasma (at a silver concentration of 0.302%) were more than 3000 times lower than those in untreated biofilms (Akiyama et al., 1998). It was concluded that the silver compounds used had a bactericidal effect against immature (24-hour) biofilms. In a miniaturized biocide susceptibility test system, the bactericidal activity of silver nitrate was determined for biofilms of *Mycobacterium phlei* (Bardouniotis et al., 2001). The minimum concentration to kill biofilm organisms was 313 mg/L and 234 mg/L silver nitrate after exposure times of 30 min and 120 min, respectively. These observations suggest that exogenously applied silver ions can be effective against biofilms, but only at relatively high concentrations. Generally, no how long the effect lasted and how many times the treatment could be repeated with success.

5.1.5.5. Surface-bound biocides and "activated surfaces"

The approach to bind biocides to surfaces in order to prevent the development of biofilms seems to be very plausible at first glance, and some authors have invented and patented systems which either carry covalently bound biocides on surfaces or on which biocides are generated. Anti-fouling paints on ships fall into this category in that they rely on the controlled release of biocides from surfaces. An old example is copper plating in order to prevent fouling of ship hulls. The release of silver from surfaces belongs in a similar category, albeit not applied to ship hulls, and is discussed earlier. These examples demonstrate the principal problems of such approaches:

- i. After a certain period of time, a natural selection process will yield strains which overcome the biocidal effect. In the case of copper, the first colonizers will be organisms tolerant to copper and may be later, in turn, colonized by copper-sensitive organisms.
- ii. The biocide will be released into the environment and can result in harmful effects. An example is tributyltin anti-fouling compounds. However, these are so toxic to marine organisms that they have been widely banned from use.

5.1.5.5.1. Surface-bound biocides. Speier and Malek (1981) report that organosilicon-substituted organic amines, amine salts [II, 18.1.10.], or quaternary ammonium salts [II, 18.1.] displayed very effective antimicrobial activity when incorporated into solids. These authors did not claim that the biocide was insoluble but rather bound by specific adsorption to negatively charged groups on glass. They gave no information about how long the effect lasted or the capacity of the surface.

Hüttinger (1987, 1988) has presented a system in which biocides such as aldehydes, hydroxylaniline, aminophenylmercuryacetate, dibutyltinchloride and tributyltinchloride [II, 19.6.] were bound to cellulose as substratum, referring to earlier work of Isquith and McCollum (1978). The efficacy was evaluated by agar plate tests. From his results he concludes that only hydroxylaniline and tetrachlorobenzene, which were not active, had to be transported into the cytoplasm to be active. However, his study did not reveal whether this was only an effect of release of the other biocides into the medium which was less pronounced with the substances mentioned as ineffective. As plausible as this idea may appear in the first place, the approach must overcome a few serious problems:

1. If no biocide molecules are actually released, it is hard to understand how molecules which do not penetrate the cell wall can kill a microorganism. An explanation was not provided.
2. If cells attach a surface impregnated with biocides and are actually killed, there is not much probability that they leave the surface. Thus, the surface will be soon covered with dead bacteria and lose its efficacy.
3. Not only can bacteria adhere to the surface but also macromolecules of all kind. They may kinetically compete with adhering cells and mask the active groups, an effect which is called "fouling".
4. The authors claim a killing efficacy against planktonic cells also which seems to be a clear indication that the biocide must have been released into solution, as a remote biocidal effect of biocides covalently bound to surfaces conferred to suspended organisms is scientifically not understandable and it was not discussed by the authors.

Tiller et al. (2001, 2002) have covalently attached poly(4-vinyl-*N*-alkylpyridinium bromide) to glass slides and, thus, created a surface which supposedly kills airborne bacteria on contact. The authors do not provide evidence about the mechanism of action. It is quite probable that traces of the biocide are dissolved into the water droplets in which the test organisms have been sprayed onto the surface. Killing rates up to 99% are reported, which cannot, however, be considered as sufficient, as they include only two log steps.

5.1.5.5.2. Natural Anti-fouling Compounds. An interesting approach is to incorporate mechanisms with which living organisms defend themselves against colonization (Wahl, 1989). Natural anti-fouling compounds have been isolated mainly from some marine plants which are not colonized by bacteria (Terlezzi, 2000). Steinberg et al. (1997) have isolated signalling molecules antagonists (halogenated furanones) from an Australian seaweed which exhibits anti-colonizing activity. More marine antifouling products have been investigated by Armstrong et al. (2000). The problem with all of these compounds is that most of them are only scarcely available, that they are difficult to apply on a constant basis on a surface and that they will select for organisms which can overcome the effect. Apart from that, they will have to undergo the EU biocide guideline procedure which is assessed to cost currently about 5 million Euro per substance.

5.1.5.5.3. Surface-Generated Biocides. Another creative approach is the actual generation of biocides at a surface after which the biocide is released. Wood et al., (1996, 1997, 1998) observed that the activity of hydrogen peroxide or of potassium monopersulphate against attached bacteria was strongly enhanced if copper or cobalt phthalocyanine was incorporated into the surfaces, acting as catalysts for the formation of active oxygen species. The authors assume that the generation of these species at the substratum-biofilm interface concentrates the antimicrobial effect to the interfacial cells and generates a diffusion pump which further provides active species to the biofilm matrix. The survivors of low-concentration treatments with these agents were more readily removed from the catalyst-containing disks than from control disks. This indicated advantages gained in hygienic cleansing of such modified surfaces. This approach was expanded to thick biofilms (100 µm) of *P. aeruginosa* and still yielded significant enhancement of killing which originates from the interface. Reaction-diffusion limitation seems to concentrate the active species within the biofilm rather than protecting it, which could explain the efficacy against thick biofilms. A further advantage may be that EPS molecules can be disrupted, thus weakening the adhesion to the substratum and leading to biofilm detachment.

5.1.6. Conclusions

The “real world” which biocides encounter when employed will be biofilms, not suspended pure cultures, because this is the preferred mode of life of microorganisms. In biofilms, they have developed many ways to increase their resistance, as discussed earlier. From an ecological view, this makes perfect sense as the organisms have had to defend themselves against adverse conditions since the evolution of life. The protection mechanisms evolved during this time had to deal with all kinds of biocidal effects as occurring under natural conditions. Therefore it is not surprising that man-made biocides usually work better in the laboratory than under practical conditions. The possibility of enhanced resistance of biofilm organisms thus always has to be considered.

It is impossible to provide detailed and predictable protocols for disinfection of biofilms in various situations which can be generalized. In principle, biocides can be effective against biofilms. However, this has to be verified in given situations and it has to be taken into consideration that the three crucial factors for biocide efficacy, i.e., concentration, exposure time and temperature, have to be optimised in order to achieve the required efficacy. It will be important to verify the effect. In order to do so, it is necessary to perform the assessment with intact biofilms and not with removed and suspended biofilm organisms as these will be much more susceptible to the biocide.

Another important aspect is to distinguish between disinfection and cleaning. Biocides are designed for killing microorganisms. However, killing the organisms and leaving the biomass in place will in most cases not solve the problem but rather lead to rapid aftergrowth. The fact that some of them actually disrupt biofilms and contribute to their removal cannot be extrapolated to all biocides.

These considerations may explain why the application of biocides in technical systems is sometimes not successful. In order to achieve success, integrated approaches (Cloete and Broezel, 2002; Flemming, 2002) are recommended. These include:

- i. Detection of biofilms on surfaces
- ii. Application of biocides and cleaners
- iii. Verification of killing efficacy and removal of biofilms

Interestingly, practically all studies on disinfection of biofilms focus on already existing biofilms and not on their prevention. Biocides can be effective in prevention of biofilm formation, as practical experience with chlorine in swimming pools proves. This aspect has not been well covered scientifically.

For optimizing the use of biocides against biofilms, it will be useful to monitor their development on surfaces *in situ*, on line, in real time and possibly in a way which can be automated. Some devices have been developed which fulfil these requirements (Flemming, 2003; Tamachkiarow and Flemming, 2003).

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5.2 Microbiological control in cooling water systems

M. LUDENSKY

5.2.1 Introduction

One of the primary problems affecting efficient operation of cooling water system is accumulation and excessive growth of microorganisms. In the operating plant, biological fouling may severely affect process equipment, leading to a progressive reduction in performance and efficiency.

Cooling water systems usually provide optimum conditions for the microbial growth. Ranges of temperature and pH, continuous aeration as well as an abundance of organic and inorganic nutrients and sunlight make cooling towers an ideal place for proliferation of many biological species. As a result, diverse microbial populations may develop.

A number of associated problems can occur due to biological factors in cooling systems, including:

- Reduction of heat transfer in heat exchangers due to slime and biofilm formation;
- Restriction of water circulation in condenser tubes;
- Plugging tubes in heat exchangers causing shutdowns and loss of production in power utilities;
- Clogging of water lines in cooling water system due to algal mats;
- Pitting corrosion of metals caused by MIC;
- Biodeterioration of wooden components of cooling towers;
- Spread of Legionnaires Disease

The main source of microbiological contamination is usually the make-up water from the water source (lakes, rivers, wells, ocean, etc). Another source of contamination is an atmosphere. Many microorganisms are removed from air when it is washed with water in the cooling tower.

Biological problems in cooling water systems are usually site-specific depending both on biological aspects of the cooling water and environmental and technological conditions in a particular system. Each cooling water system presents a unique combination of equipment, water chemistry, water microbiology, contaminants, temperature, blowdown, make-up, etc. Monitoring of cooling water parameters is an essential part of the process of selection of cooling water treatment measures. Control of biological problems in cooling water needs a multifaceted approach including selection of cooling tower design, proper construction materials, cooling system maintenance, application of biocides/microbicides, condenser tubes cleaning, etc. The primary method of biofouling control is usually proper biomonitoring and application of appropriate cooling water biocides/antimicrobials. The choice of biocidal program is determined by many factors, but three stand out above all others: efficiency, cost and environmental acceptability. These and other related aspects of biocide implementation in cooling water system will be reviewed in the current chapter.

5.2.2 Cooling water systems

Cooling water systems are critical components of most major industries, including energy generating, general manufacturing, oil production, chemicals, steel, metalworking, etc. The main function of a cooling system is to remove heat from a process or equipment. Most often the cooling medium is water. Appliances that have to be cooled include condensers and heat exchangers, oil, air, gas and liquid coolers, motors and compressors, furnaces, rolling mills, chemical reactors, etc. The cold water enters these appliances and is heated by contact with hot surfaces. At the outlet the heated water can be handled in one of three ways: 1) the hot water is discharged into a river or other receiving body (open once-through cooling system); 2) hot water is cooled by contact with secondary fluid and is returned to the appliance without coming into contact with the atmosphere (closed system); 3) hot water is cooled in a cooling tower through partial evaporative condensation and then returned to the appliances (open recirculating water system). The systems of the third type are used most often. During evaporation at the cooling tower the system loses some pure water which initiates an increase in concentration of the remaining dissolved solids. In order to control this concentration some water must be removed from the system (blowdown) and replenished with fresh water from the water source (make-up). The ratio of the make-up to the blowdown is called “cycles of concentration”, which is a very important parameter in cooling water treatment practices relating to the level of closure of the system.

What is happening in cooling water? In correlation with cycles of concentration, dissolved solids concentrate, which lead to corrosion and deposition problems. Deposition is based on two mechanisms – scaling and fouling.

Scaling is a formation of minerals coming out of solution, while fouling is a result of insoluble materials of organic or inorganic nature present in the cooling water: clays, silts, mud, iron oxide, process leaks, organic materials coming from fresh water, ground water, technological process or from the intake air. An important component of fouling is biological fouling. A wide range of organisms are found to colonize cooling water systems: microorganisms colonize heat exchangers and other surfaces, the resulting layers of slime reduce heat exchange efficiency thereby increasing generating costs. Anaerobic conditions under layers of slime stimulate corrosion. Macro-invertebrates usually colonize the intake structures, cooling water intake tunnels, screens, and condenser tube plates.

5.2.3 Problems caused by biofouling

Biofouling, in general, refers to the deposition and growth of living matter in cooling water systems (surfaces and bulk water) and may be divided into two groups: (1) Microfouling, including bacteria, algae and fungi; (2) Macrofouling, including mussels, barnacles, hydroids.

Microbiology of the cooling water is not the focus of this chapter, therefore, the description of the biological fouling here will be limited to the organisms and biological processes that severely affect cooling water systems. An excellent general review of microbiology of cooling water was published earlier (McCoy, 1980). An open recirculating cooling water system with a typical localization of biofouling components is shown in Figure 1.

Biofilms

The list of most commonly found bacteria in cooling water, conditions for their growth and problems caused can be found in Table 1. It is necessary to emphasize, however, that the most damage microorganisms cause while concentrating on surfaces. There are several definitions of microbial growth on the surfaces including fouling, microfouling, slime, biofilms, etc. Due to significant developments in biofilm research in recent years, industry has almost completely embraced biofilms as a source of microbiological problems in most applications. In any case, it is becoming increasingly clear that biofilms rather than planktonic microorganisms cause essential damage to the water based processes. Therefore, we will use the word "biofilm" as a definition of a microbiological component of fouling in cooling water systems. It is almost impossible to talk about biofilm-related problems in cooling water systems in general. The properties of biofilm vary with specifics of cooling water system, as well as with environmental factors such as surface materials, nutrient conditions, hydrodynamics, source of microbiological contamination, species distribution, etc. (Characklis and Marshall, 1990; Morck et al., 2001). From practical point of view biofilm is a complex dynamic organic polymer structure, which is developed and constantly changed by community of living in it microorganisms. There are at least three features (properties) of the biofilm that effect the environment and normal functioning of industrial technological process: physical (structural), chemical (metabolic) and biological (living). Each of these biofilm elements can effect

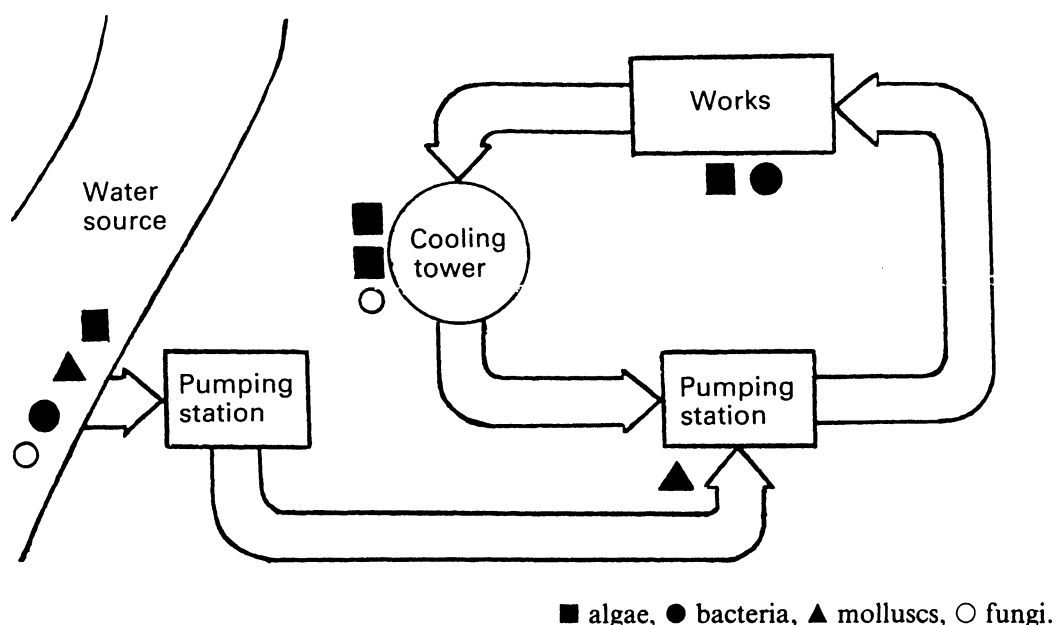


Figure 1 Diagram of recirculating cooling water system.

Table 1 Characteristics of microfouling in cooling water.

Growth type	Examples	Growth conditions		Location	Problems
		Temperature°C	pH		
Bacteria	<i>Aerobacter</i>	15–65	4–9	Condenser tubes,	Reduction in heat
Slime-forming	<i>Flavobacterium</i>			pipes, water lines	exchange, corrosion
	<i>Pseudomonas</i>				
Spore-forming	<i>Bacillus</i>	15–65	5–9	Various locations	Produced spores are
					difficult to kill
Filamentous	<i>Sphaerotilus</i>	15–45	5.5–9	Condenser tubes	Reduce cooling efficiency
Iron	<i>Galionella</i>	10–30	5.5–8	Piping, water lines	Forms slime deposits
	<i>Crenothrix</i>				
	<i>Leptothrix</i>				
Sulfur aerobic	<i>Thiobacillus</i>	15–40	1–6	Water lines, piping	Produce acid, corrosion
Sulfate reducing	<i>Desulfovibrio</i>	25–60	4.5–8.5	Anaerobic locations	Initiates MIC
Algae	<i>Stigeoclonium</i>	15–50	5.5–9.5	Cooling towers	Problems with
Green	<i>Ulotrix</i>				water distribution
Blue-green	<i>Oscillatoria</i>	20–55	5.5–9.5	Cooling towers	Reduce cooling
	<i>Phormidium</i>				efficiency
	<i>Lyngbya</i>				
Diatoms	<i>Nitzschia</i>	10–45	5.5–9.5	Cooling towers	Biocides adsorption
	<i>Navicula</i>				
	<i>Fragilaria</i>				
Fungi	<i>Aspegillus</i>	0–40	2–8	Cooling towers	White or brown rot of
Filamentous mold	<i>Penicillium</i>				cooling tower wood
	<i>Fusarium</i>				
Yeasts	<i>Saccharomyces</i>	0–40	2–8	Cooling towers	Slime formation
	<i>Torula</i>				
Basidiomycetes	<i>Lenzites</i>	0–40	2–8	Cooling towers	Internal wood decay

industrial technological process. For example, biofilm structure of bacterial extracellular polymers may cause an unusually high fluid frictional resistance in water conduits, metabolic activity within biofilm may change chemistry of the environment and initiate microbiologically induced corrosion or odor problems. On the other hand, being a consortium of living microorganisms, biofilm serves as a depot of potential contamination problems or infections. See also chapter 5.1.

In a recirculating cooling tower system microbial growth could be very high due to the presence of nutrients, favorable temperatures, high residence time, high ratio of surface area to the volume, etc. In general, a cooling tower is an ideal place for the growth of living organisms, because it provides air, heat and light. The major economic impact caused by biofilms in cooling water systems is because of energy losses due to increased fluid frictional resistance and increased heat transfer resistance at power plant condensers and process heat exchangers. Biofilms accumulating on the surfaces of heat exchange tubes significantly reduce the heat transfer rate because the thermal conductivity of biofilms is significantly less than that of metal heat transfer surface materials. Other negative impacts of fouling include increased capital costs for excess equipment capacity and premature replacement of equipment experiencing MIC, unscheduled turnarounds or downtime to clean equipment that fouled, as well as safety problems, including *Legionella* related problems (Kemmer, 1988; BetzDearborn Handbook, 1991; Drew principles, 1994; Bott, 1998).

Legionella

Following the 1976 American Legion Convention at the Bellevue Stratford Hotel in Philadelphia, 34 attendees died and 221 people became ill from Pneumonia caused by the bacterium *Legionella pneumophila*. Since that time subsequent outbreaks and sporadic cases of Legionellosis have occurred all over the world. In a number of cases cooling water systems played a key role in disseminating of this bacterium. It is clear now that a cooling tower can present an ideal environment for growth of *Legionella pneumophila*. The formation of a biofilm within a water system is thought to play an important role in harboring and providing favorable conditions for *Legionella pneumophila* proliferation. It grows within biofilms and within protozoa acting to shield *Legionella pneumophila* from biocides that would otherwise kill freely suspended bacteria. Cooling tower drift in the form of aerosols can be easily inhaled or carried toward the air inlets to the air conditioning systems of nearby building or facilities. If cooling water contain any amount of planktonic *Legionella pneumophila* the disease can be initiated. Even one single organism can be the cause of disease in a susceptible individual. Multiple studies conducted by CDC and research institutions did not show any special pattern of *Legionella* resistance to commercial biocides (McCall et al., 1999; Thomas et al., 1999, Lin et al., 2001). However, due to the presence of biofilms in cooling water systems, there is no known practical level of chemical treatment that effectively keeps *Legionella pneumophila* out of cooling systems. To remove all risk,

complete elimination of the organism must be achieved, which is not practically possible due to the constant reinoculation of cooling systems with bacteria, as well as due to economic and environmental constraints. Currently EPA does not allow any claims for water treatment products that imply control of *Legionella*. EPA's rationale is based on the fact that there is no published criteria or standard for a "safe" *Legionella* level in water systems and that complete kill in such systems is unlikely. Many research and industrial organizations including CDC, ASHRAE, CTI, NACE have issued guidelines or statements addressing *Legionella* problems and practices for its control (CTI publication, 2000; ASHRAE publication, 2000). They advise to implement an effective microbiological control program to reduce the risk. Such program recommends to include routine treatment of cooling water with oxidizing or non-oxidizing biocides that would provide the most effective slime and algae control for a particular system along with periodic on-line disinfection in the systems where *Legionella* test results show greater than 100 cfu/ml.

Algae

Algae are photosynthetic organisms that need sunlight for their metabolism. Because their living requirements are within the ranges of conditions in basic cooling water systems it is not surprising that they often proliferate in cooling towers. They can produce prolific algae blooms in the water or may grow as free-floating slime masses on the water surface. More often, however, they are attached to the wetted surfaces of a cooling tower exposed to air and light.

When abundant in cooling water towers and spray ponds, algae may disrupt water distribution, stimulate biocorrosion, block screens and distribution decks. Severe algae fouling can lead to unbalanced water flow and reduced cooling tower efficiency. A review of algal fouling in cooling water systems can be found in (Ludyanskiy, 1991). It was found that the algal flora is greatly influenced by the conditions in the cooling systems as well as by geography of location. The temperature and chemistry of the water were noted to affect the biomass and abundance of species more than any other factor. For example, in the study of algal fouling at several industrial cooling systems in Ukraine it was demonstrated that an increase in water temperature to 30°C or more resulted in the shift of algal microflora towards blue-green algae domination, since these have a wider range of tolerance. It was further noted that: (1) the biomass dynamics of algal fouling was strictly seasonal; (2) there was a strong dependence between the algal flora in cooling water systems and the river sources; (3) diatoms are usually present in most cooling towers but generally do not cause problems in cooling water systems.

Fungi

The main concern of fungi growth in cooling water systems is the effect of fungi on wood components of cooling towers. Table 1 summarizes some of the important conditions of fungi growth. The fungal attack could be of three types: (1) soft rot – occurs primarily on the surface of wetted wood and can be found most commonly in the fill section of the cooling tower and is caused by fungi metabolizing cellulose; (2) brown rot – occurs primarily inside wood that is not fully saturated with water, mostly in mist-area wood, may be caused by a combination of filamentous mold and basidiomycetes; (3) white rot produces cavities inside the wood caused by basidiomycetes, which can attack cellulose as well as lignin and most other wood components, all the wood components in an area may be digested, creating a hollow cavity. All in all, fungi can be quite troublesome, causing severe damage to the wooden components of cooling towers, colonizing wood surfaces and producing bacterial-like slimes.

Macrofouling

Macrofouling consists of macro-invertebrates such as clams, mollusks, barnacles, oysters, bryozoans, together with fish and vegetable matter such as seaweeds and grasses, dead vegetation and man-made debris. It is the living matter that is generally regarded as macrofouling. These deposits are more likely to be manifested in once-through cooling systems employing marine, estuarine or river water, and it is likely to occur at intakes of power plant cooling water systems. Extended reviews on the biology of macrofouling were published earlier: marine fouling (Whitehouse, et al., 1985); Corbicula (McMahon, 1983); Zebra mussel (Ludyanskiy et al., 1993)

Macrofouling of plant cooling water systems by marine and fresh water macro-invertebrates costs industry billions of dollars each year. These problems include development of heavy thick encrusting growth in the intake structures of cooling water systems, reducing water flow, initiating periodic sloughing of mollusk shells and clogging screens and condenser tubes. These problems may be extremely costly to the electric power industry, especially at the nuclear plants, where it may affect integrity and safety of operation. In marine and estuarine environments, macrofouling of plant water systems is common. Many organisms capable of causing macrofouling problems have been identified as potential problems. The most costly and troublesome organisms are the shell-forming organisms, first of all barnacles and mussels. Especially problematic species are acorn barnacles, such as *Balanus improvisus*, and mollusks, such as *Mytilus edulis*. The most important feature of life cycle of

both organisms is the fact that they release very small free-swimming larvae, which pass through intake water screens and settle in various places in intake structures where they grow to adult stage. Barnacle larvae is capable to attach even at very high water velocities up to 3 m/s. *Mytilus edulis* prefers low flow conditions. For both organisms, the shell of the adult presents the main problem. The effect from the barnacle fouling comes from settlement on valve facings and filter meshes. The barnacle structure will remain attached for long time, even after the death of the organisms, while the shells of dead mollusks can slough and plug condenser tubes.

There is a much lower diversity of macrofouling organisms in freshwater environments. Therefore, freshwater macrofouling problems are more regional and sporadic. Problems, however, can be particularly severe including episodes of plant shutdowns. Of freshwater macrofouling organisms, bivalve mollusks *Corbicula fluminea* (Asiatic clam) and *Dreissena polymorpha* (Zebra mussel) have the most impact on cooling water systems. Introduction of these two species to North American water bodies occurred in 20th century (Asiatic clam in 1930s, and Zebra mussel – in 1980s). Due to a lack of natural predators and a suitable environment, both species has since spread dramatically. As is the case with the marine mussels, freshwater bivalves release mobile larvae (veligers) at specific for each species temperature, which then pass through the screens and settle on various surfaces. Upon maturation, they develop clusters of shells which bring significant problems. The comparison of most important parameters of mollusks development and distribution is shown in Table 2.

5.2.4 Methods of testing and evaluation

Identification of planktonic organisms

There are many methods to identify directly or indirectly the level of microbial fouling in cooling water systems. The simplest (and the most used) methods are based on the measurement of the total number of microorganisms in the water. The most often used method is the total bacterial and fungal plate counts of the samples of cooling water. For bacteria, Tryptone Glucose Extract Agar is used as a growth medium, incubation usually takes 48 hours at 25°C or 25 hours at 37°C. Fungi are usually cultured for 5 days at 25°C in Sabouraud Dextrose Agar. There are special plate count techniques for determination algae, SRBs, and other biological organisms. Newer techniques, including dip slides of different kinds were developed to simplify field testing. It must be emphasized that not all bacteria can grow on selected growth media. Therefore, results of plate counts and dip slides could supply only semi-quantitative data.

Many studies were performed with the use of plate count techniques. Experience demonstrates that the concentration of microorganisms in the cooling water depends on many parameters and is site-specific. However, the normal functioning of cooling water system is reported when bacterial counts are below 10⁵ colony-forming units (cfu) per milliliter of cooling water in open recirculating systems, 10⁴ cfu/ml in closed systems or 400 cfu/ml in a makeup supply (Drew, 1994).

The same techniques are usually performed to evaluate effectiveness of biocides for cooling water applications. For example, ASTM E 645-97 is usually used to determine efficacy of microbicides for controlling microbial growth in cooling towers using cooling water collected from operating cooling water systems.

However, the results obtained by this methodology may not be predictive of what will occur in the field. The reasons for this is that microorganisms causing the biofilm formation in the field are more resistant under field conditions or that they are not recoverable on the medium used to enumerate planktonic microorganisms. Other methods for assessing efficacy of the microbicide against should be utilized.

Biofilm monitoring

It is now widely recognized that sessile microorganisms cause the most damage in industrial water systems, and the development of biocides is directed towards biofilm. In order to measure the effectiveness of a biofilm control program, it is essential to accurately monitor the development of biofilms in a system and record their response to control measures.

Table 2 Characteristics of macrofouling in cooling water

Mollusk type	Min. Spawning Temp., °C	Adult shell length, cm	Life span, years	Planktonic period, days	Settlement seasons	Larval size, µm
<i>Corbicula fluminea</i>	5	1–4	6–8	Accidental	Spring and fall	18–25
<i>Dreissena polymorpha</i>	12–14	3–5	4–8	8–16	May–Oct	400–200
<i>Mytilus edulis</i>	8–10	5–9	2–16	20–60	May–Sept	70–260

The relevant scientific literature contains hundreds of papers describing methods that have been successfully used to grow and measure biofilms. Since biofilms may form in many different types of systems, no one method can be presented that evaluates all the factors affecting biofilm formation. That is why an ASTM "Standard guide for selecting test methods to determine the effectiveness of antimicrobial agents and other chemicals for the prevention, inactivation and removal of biofilm" (ASTM E 1427-00, 2000) lists several dozens methods that can be used for biofilm formation and measurement. There are many more methods suggested in recent reviews on biofilms, microfouling and on evaluation of monitoring techniques (Characklis and Marshall, 1990; Warwood et al., 1991; Brown and Gilbert, 1995; Flemming and Schaule, 1996, Ceri et al., 2001). Success in selecting test methods depends on the understanding of which criteria are most important in a particular system and the ability to simulate applicable field condition. Methods that are used in biofilm screening systems to assess biocide efficacy on biofilms include measurements of slime weight and thickness, viable and total cell counts, protein content, organic carbon, muramic acid, INT, ATP production, catalase activity, hydrolase activity, DNA, lipids, respiratory activity, among many others methods to measure biofilm accumulation or microbial activity. The recent tendency in biofilm research has been to go "high-tech" using sophisticated techniques in order to understand structure, functions and response of biofilms on the microlevel, including confocal laser microscopy, spectrochemical, biochemical, electrochemical, molecular, and imaging techniques. However, in industrial applications, accurate information on the relative efficacy of biocides on biofilms is considered sufficient in most cases. Industrial microbiologists have selected several indirect techniques for biofilm sampling, biocide screening and biofouling monitoring based on the decision to study the biofilm itself and not individual organisms within the film. That is why, the use of fouling monitors in industrial applications focuses on several indirect methods incorporating changes in: 1) heat transfer resistance, 2) fluid frictional resistance (differential pressure), 3) optical attenuation. The review covering the current status of industrial biofilm monitoring techniques was published recently (Ludensky, 1999). Table 3 presents the list of biofouling monitors and biofilm sampling devices developed in the last two decades.

Current biofilm screening and monitoring techniques that are used in aquatic industrial applications hardly satisfy the requirements of industry in measuring performance efficacy of biocidal products and monitoring fouling in cooling water systems. Experimental approaches used for laboratory screening of biocides vary significantly. Each of the various testing methods uses different procedures to establish and maintain the test biofilms. Accordingly, each approach produces biofilms that differ from one another in terms of morphology, physiology and metabolism. These factors undoubtedly have a significant impact on the efficacy of the biocides tested. Therefore, the comparison of experimental data on efficacy testing of biocides conducted in different laboratories might differ significantly.

As for the field monitoring of biofilms, there is a need for a rapid method of quantification of biofilm presence in the system (more reproducible than ATP measurements). Plate counts and other traditional methods that are still used to measure microbiological activity in industrial systems are slow, labor intensive, and often not representative of sessile growth.

5.2.5 Biocides in cooling water systems

Selection of a biocidal program

Selection of a biocidal program is not an easy task. Generally speaking, given sufficient concentration and contact time, virtually any biocide available for cooling water systems can control biological fouling. The question is to find measures of biological control that would be the most cost-effective in a particular cooling water system,

Table 3 Commercial fouling monitors and biofilm sampling devices

Robbins Device from Tyler Instruments (coupon collection);
Betz BioBox (coupon collection);
Betz Biofilm Fouling Monitor (coupon collection);
Bio-Fouling Monitor from Yost and Son (coupon collection);
Biofilm Probe from Caproco (coupon collection);
Dats Fouling Monitor from Bridger Scientific (heat transfer resistance);
PDM-1 Deposition Monitor from Yost and Son Inc. (heat transfer resistance);
Cortest Heat Exchanger System from Cortest (pressure drop and heat transfer);
Nalco Delta-T Biofouling Monitor (Pressure drop measurement);
RotoTorque from Montana State University (fluid frictional resistance);
Laboratory Biofilm Reactor System from Biosurface Technology. Corp.;
MBEC TM Device from MBEC Biofilm Technology;
Biofilm Development Assemblies from PS Biofilm Technology;
Rotating Disc Reactor from Montana State University;
Biofilm optical monitors from Ondeo NALCO

while satisfying safety, environmental and legislative requirements. Analysis of parameters important in selection of biocides was previously published (Freedman, 1979; EPRI Handbook, 1993; Flemming and Schaule, 1996).

Setting up a control program involves consideration of at least three major factors within treated cooling water system

1. Type, size and condition of the cooling water system. The level of the system closure effect significantly biocide choices. For example, discharges of once-through cooling water systems are strictly regulated, therefore low levels of oxidants are the usual choice. Continuous windblown debris can accumulate throughout the cooling water system, and can rapidly adsorb some biocides. If the system is heavily fouled, adding of any biocide will not help much until system will be cleaned.
2. Water quality factors. Such parameters of cooling water as pH, temperature, organic load, presence of ammonia, corrosion and scale inhibitors, reducing agents can greatly influence biocidal activity of biocides and will help in selection of the most appropriate agent for a particular system. A process leak in the system is another significant factor that might interfere with biocide chemistry thus effecting biocontrol efficacy.
3. Water discharge limitations. These issues are based on federal, state and local regulations, and are always a major factor in biocide selection, having impact on frequency of biocide application, dosage level, etc.

Selection of a biocidal program depends largely on the particularities of specific biocides:

- Microbicidal efficacy in cooling waters
- Mechanism of biocidal efficacy
- Spectrum of activity
- Feeding specifics
- Stability in cooling water
- Compatibility with other cooling water additives
- Corrosive effects on the system
- General toxicity
- Safety and health aspects of handling and storage
- Biodegradability
- Analytical detection and monitoring
- Effect on the organisms in sewage water

All in all, it is clear that the selection of biocidal program needs a highly specialized expertise. It is not surprising, therefore, that this is mostly performed by water treatment service companies.

Cooling water biocides

Cooling water biocides can be classified in two different ways: (1) by target organisms; and (2) by chemical activity. When classified by target organism, cooling water biocides can be described as bactericides, algacides, fungicides or molluscicides. More often, biocides are classified by chemical activity as oxidizing or non-oxidizing.

*Oxidizing biocides [II, 21.]**

Class of oxidizing biocides includes chemicals that function by halogenating macromolecules within a cell, including chlorine, bromine and many different types of halogenated compounds (sodium bromide, stabilized bromine products, halogenated halohydantoin, chlorinated isocyanuric acids, etc.) Oxidants also include halogenated or non-halogenated chemicals that function by generating free radicals (chlorine dioxide, hydrogen peroxide, peracetic acid). Moderate to high concentrations of oxidants can “burn” through most biological material, reacting non-specifically with the nucleophilic functional groups of proteins, lipids, carbohydrates and nucleic acids (Weincek and Chapman, 1999). Oxidizing biocides are very effective against most problem organisms, reacting readily with the general protoplasm of bacteria, and exhibiting rapid speed of kill. Oxidizers are used more often than other classes of biocides in cooling water systems. This is because they are less persistent in the water, less toxic to the environment and people, as well as they are safer to handle and more convenient to use. Historically, there was continuous trend for use of cooling water biocides from more toxic to less toxic due to tightening regulatory requirements. Another significant impact is due to many fundamental changes in cooling water conditions, chemistry and treatment. Current cooling water parameters are (Puckorius, 1999a):

- Higher pH cooling water: 7.5–9.5;
- Higher levels of alkalinity: 150–500 ppm;
- Total hardness much higher: 1000–3000 ppm;

*see Part Two – Microbicide Data

- Higher cycles of concentration: 8–20;
- Significant changes to the film fill design.

Oxidizing biocides can be classified in two different ways: (1) by form of the product or (2) by active chemical. Oxidizing biocides are available as powders, gases, liquids, granules, tablets, briquettes of various sizes and solubility characteristics. By active chemical, cooling water oxidizers can be divided in several groups:

Chlorine based oxidants;
 Bromine based oxidants;
 Chlorine/bromine combinations;
 Chlorine dioxide products;
 Organic halogen products
 Non-halogen oxidants.

Major oxidizing biocides are characterized in Table 4, which is based on the information from available technical literature (BetzDearborn Handbook, 1991; CTI Publication, 1994; Drew principles, 1994; Bartolomew, 1998; Puckorius, 1999b).

Halogen biocides are, by far, the most often used type of oxidizing biocides. Efficacy of halogen-based oxidizing biocide depends significantly on the halogen species of biocides and halogen demand of the cooling water. When added to cooling water halogen undergoes a variety of reactions with contaminants and constituents of the water. Consumption of halogen in the water leading to non-biocidal ingredients is referred to as the halogen demand. Some of created in the reaction constituents may be as biocidal as biocides themselves. Several cooling water components react with all oxidants and may reduce biocidal effectiveness: nitrogen compounds, hydrocarbons, iron, manganese, sulfides, as well as silt, vegetation, algae and slime. Free oxidant

Table 4 Main properties of oxidizing biocides

Biocide type	Form	Incompatibilities	Strength	Weakness
Cl ₂ [II, 21.2.1.]	Gas	Less effective at high pH, in presence of ammonia, sunlight, etc.	Economical, effective	Toxic gas Safety concerns Costly equipment
NaOCl [II, 21.2.2a.]	Liquid	Less effective at high pH, in presence of ammonia, sunlight, etc.	Economical, effective	Low activity, limited stability
Calcium hypochlorite [II, 21.2.2b.]	Solid	Less effective at high pH, in presence of ammonia, sunlight, etc.	Economical, effective	Adds calcium to the system
NaOCl + NaBr [II, 21.2.3.]	Liquid	As other chlorine and bromine species attacks phosphonates, nitrites, azoles, but not inorganics	Effective, easily retrofitted to most chlorination systems	Requires feeding two chemicals
Bromine Chloride	Liquid	Attacks phosphonates, nitrites, azoles, but not inorganics	Most economical form of bromine	Feeder cost, safety and handling concerns
BCDMH [II, 21.2.11.]	Solid	Less effective at pH > 9 and at high halogen demand	Higher safety Easy to handle	Higher cost, storage, feeder issues
BCDMH/BrMEH	Solid	Less effective at pH > 9 and at high halogen demand	Higher safety Easy to handle	Higher cost, storage, feeder issues
DCDMH [II, 21.2.10.]	Solid	Attacks phosphonates, nitrites, azoles, but not inorganics, less effective at pH > 7.5	Higher safety Easy to handle	Higher cost, storage, feeder issues
Stabilized bromine	Liquid	Attacks some additives, but less than chlorine, Degrades at high temp.	Good stability, less toxic Liquid bromine product	Higher cost, less effective on algae and at high organic load
Chlorine dioxide [II, 21.2.4.]	Gas	Cooling water additives, mostly organics, Does not attack most inorganics	Effective at high organic loading, in presence of ammonia, and at high pH	Needs to be generated on site, high volatility, safety concerns
Peracetic acid [II, 21.1.3.]	Liquid	Will attack most inhibitors	Broad spectrum, Biodegradable	Corrosive
Hydrogen peroxide [II, 21.1.1.]	Liquid	Will attack most inhibitors, inactivated by catalase	Degrades to water and oxygen	Slower acting, needs higher residual
Chlorinated cyanuric acid derivatives [II, 21.2.6., + 7.]	Solid	Attack phosphonates, nitrites, azoles, but not inorganics	Added stability Higher safety Easy to handle	Higher cost
Ozone	Gas	Can attack almost all additives, PVC, copper, wood and gaskets	Effective against spore-formers and fungi	Very reactive and volatile, corrosive, rapidly lost, low solubility, safety concerns

residuals can be degraded rapidly upon exposure to UV light and high temperature. Therefore, selection of oxidizing biocide is also very site-specific. In general, chlorine is the most popular biocide due to its cost efficacy. However, bromine compounds are gaining momentum in the past two decades due to some technical advantages. For example, chlorine and bromine react with nitrogen impurities, such as amines, urea, proteins, and amino acids in a different way. Combined forms of chlorine (chloramines) are generally less effective biocides than free chlorine by a factor 7-10 or more. On the other hand, bromamines, combined forms of bromine, are mostly effective biocides. Therefore, it is common practice to use bromine compounds in presence of ammonia in cooling water. Another advantage of bromine in current cooling water systems is its high efficacy at pH above 7.5, where chlorine is less effective. Solid organic halogen release biocides, halohydrantoin, are considered to be the top-of-the-line oxidizing biocides, combining high biocidal efficacy with safety, ease of handling and use (Drew principles, 1994; Ludyanskiy and Himpler, 1997; Bartholomew, 1998).

Non-oxidizing biocides

In spite of high effectiveness of oxidizing biocides in cooling water systems, it is sometimes difficult to control problem organisms just with oxidizers alone. High reactivity and low persistence of most of oxidizers can leave some microorganisms unharmed, especially those that proliferate in biofilms. Therefore, the need exists in other classes of biocides that are more persistent and aimed at organisms less effected by oxidizers.

Non-oxidizers can do some jobs better than oxidizers, and some that oxidizers can do only poorly. For example, control of algae, sulfate-reducing bacteria and fungi is generally more effective with specific non-oxidants. Major consideration in the use of non-oxidizers is their persistence in cooling water system, which correlates with the longer half-life and better efficacy. However, persistence of these biocides in environment is a negative feature (due to toxicity to fish and other aquatic organisms). Therefore, level of degradation of non-oxidizing biocides is an important factor in their application.

The modes of action of non-oxidizing biocides are very specific and rather complex (Paulus, 1993). They are discussed in the current book in detail in chapter 2 and Part Two. An excellent overview of mechanisms of action of cooling water biocides was recently published (Weincek and Chapman, 1999). Major non-oxidizing cooling water biocides are characterized in Table 5, which is based on available technical literature (BetzDearborn Handbook, 1991; Drew principles, 1994; Puckorius, 1998a, Puckorius, 1998b).

Most of non-oxidizing biocides are liquids, and they can be classified rather vaguely by mode of action, by speed of kill, by active chemical group, etc. We will address here only significant practical aspects of most commonly used non-oxidizers. An information on other non-oxidizers can be found in the recently published reviews by Puckorius and Associates (Puckorius, 1998a, Puckorius, 1998b).

Table 5 Main properties of non-oxidizing biocides

Biocide type	Target organisms	Incompatibilities	Strength	Weakness	Mechanism
Quats [II, 18.1.]	Bacteria Algae Mollusks	High suspended solids, anionics, salinity	Broad spectrum, Fast acting	Not effective vs. fungi, Foam possible	Cationic surfactant, adsorption on cell wall and cytoplasmic membrane
DBNPA [II, 17.5.]	Bacteria	Unstable at pH > 7.5	High efficacy	Not persistent at higher pH,	Inhibits respiration and enzyme activity
Isothiazolone [II, 15.]	Bacteria Algae Fungi	Unstable at pH > 9.3, sulfides	Persistent broad spectrum	Irritation, Skin sensitization	Effect respiration, enzymes and protein synthesis
Glutaraldehyde [II, 2.5.]	Bacteria Algae Fungi	Ammonia, amines, high temperature and pH	High efficacy in various applications	Inhalation toxicity, odor	Cross-links cytoplasmic membrane structure
DGH [II, 18.3.]	Bacteria Algae Fungi	High alkalinity, sulfate and phosphate	Effective at broad pH range	Reacts with anionics	Disrupts cell wall, reduces Membrane permeability
Dithiocarbamates [II, 11.10.–11.13.]	Bacteria Fungi	Cationic biocides, dissolved iron	Low cost	Cationic biocides Presence of iron	Loss of cell viability, and intracellular protein thiols
DTEA	Bacteria Fungi Algae	High turbidity	Very persistent	Not effective at pH > 8.5	Destroy functionality of metal-containing coenzymes
THPS [II, 3.6.]	Bacteria Algae	Anionics, Chlorine Bromine	Fast acting Very good toxicity profile	Reacts with anionics	Protein destruction of cell wall
Thiocyanates [II, 20.9.]	Bacteria	Rapid decomposition at pH > 7.5	Low cost	Solvent is organic Low solubility	Loss of intracellular protein thiols
BNPD [II, 17.14.]	Bacteria	High pH, Ammonia Amines	Effective against SRB	Formaldehyde releaser,	Disrupts protein synthesis

Quaternary ammonium compounds are cationic, surface active chemicals and represent organically substituted nitrogen compounds with 8 to 25 carbon atoms bound to a nitrogen atom, while the total cationic portion is associated with a halide ion. Quats belong to the class of membrane-active biocides (together with polyquats, and biguanides), that disrupt the structure and function of the cell membrane by forming an electrostatic bond with the negatively charged sites on the cell wall. Quats do not discriminate between living cell and negatively charged suspended solids, therefore their persistence is not very good. Quats are highly effective fast killing broad spectrum cost-competitive non-oxidants with good biodispersant characteristics. They are effective against bacteria, algae and mollusks. Quats activity drops in systems heavily fouled with dirt, oil and debris, and quats can cause extensive foam if overfed.

Isothiazolone (5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazoline-3-one) [II, 15.3.] is one of the more popular non-oxidizer biocides utilized in cooling water applications. Mechanism of its efficacy is based on its interaction with thiol groups, inhibiting enzyme reactions and respiration. This biocide has gained widespread acceptance because of its effectiveness at low concentrations against bacteria, fungi, and algae in a wide variety of water types across the spectrum of pH, suspended solids, and nitrogen compounds, normally encountered in cooling water systems. Since the primary mode of action is the tying up of disulfide linkages in the cell, isothiazolone may be easily neutralized if cooling water contains high levels of sulfide. Environmental characteristics of isothiazolone are also attractive due to the fact that degradation of biocide from the discharge stream may be happening via several pathways, including hydrolysis, biodegradability and photolytic breakdown. Among drawbacks of this potent biocide is a relatively slow kill, and safety concern arising from the fact of its sensitizing characteristics.

DBNPA (2,2-dibromo-3-nitrilo-propionamide) [II, 17.5.] is a potent, broad spectrum, very effective bactericide. Its mechanism of microbiocidal action falls somewhere between an oxidizer and a non-oxidizer. Best results with this biocide are seen when applied at pH below 8.0 in cooling systems with low organic contamination. At higher pH, its half-life may be too short to provide sufficient contact time with target microbes. In general, DBNPA kills in a very short period of time, then degrades almost as rapidly. Recent studies raised safety concerns when using DBNPA.

Glutaraldehyde [II, 2.5.] belongs to the class of aldehyde-based biocides that react non-specifically with cellular nucleophiles (R-NH₂, R-SH, R-OH). Glutaraldehyde contains two aldehyde groups that can interact with amino-groups in proteins, causing cross-linking within and between proteins. Glutaraldehyde is a broad spectrum biocide showing particular strength in controlling sulfate-reducing bacteria. The ability of this material to work more rapidly at high pH makes it popular choice for alkaline cooling waters. Glutaraldehyde is unaffected by oil contamination and high levels of sulfides, but shows reduced activity in the systems with high ammonia content. However, the product has three limitations: a characteristic aldehyde odor, aquatic toxicity, and recently found inhalation toxicity.

Synergistic combinations

The dynamics of microbial populations in cooling water are complex. Quite often it is difficult to achieve microbiological control with just one biocide. Some proprietary cooling water antimicrobials are formulated to contain more than one active. Proper blending of actives can compensate for limitations in spectrum of kill by one or more actives or help control resistant forms of organisms. With no increase in the amount of biocide used, the power of a blend can exceed that expected from a simple additive effect. Synergism is known as a condition where the effects produced by dosages of two different materials blended together are significantly higher than the sum of effects when materials are used individually. The synergism index was first introduced in 1961 (Kull et al., 1961), and since then has become a common parameter to measure activity of biocidal combinations. Many biocidal blends have been patented in 1980s–1990s. There is a trend now in the water treatment industry to expand capabilities of existing biocides by finding new effective combinations. Investigations usually start with active substances already in widespread commercial use, such as quats, aldehydes, isothiazolone, etc. Their action is enhanced by suitable additives. Some of the most popular biocides for blending are: 2-(Thiocyanomethylthio) benzothiazole [II, 15.11.], methylenebis(thiocyanate) [II, 20.9.1.], 5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazoline-3-one [II, 15.3.], dodecylguanidine hydrochloride, bis(trichloromethyl) sulfone [II, 17.7.], 1-bromo-1-(bromomethyl)-1,3-propanedicarbonitrile [II, 17.5.], alkyl dimethylbenzylammonium chloride [II, 18.1.2.], bis(tributyltin)oxide [II, 19.5.]; 2-bromo-2-nitropropane-1,3-diol [II, 17.14.], beta-bromo-beta-nitrostyrene [II, 17.17.] (Federally Registered pesticides, 1998). In some cases, reinforcement is obtained with substances that themselves have little or no biocidal activity, such as surfactants, complexing agents, enzymes, etc. The search for new antimicrobial agents is becoming increasingly difficult, since a broad field has already been investigated and official requirements for biocide registration are tightening. Besides, the cost of developing new molecules is very high. It is estimated that the costs of developing the required efficacy data and the other essentials of the registration process, required by law just for one product, can be above 3 million dollars (Puckorius, 1999a). See also chapter 3. In practical terms, it means that water treatment companies have

incentive to work with already established biocides trying to obtain improvements by creating new synergistic combinations. It is worth mentioning that sequential feeding of antimicrobials to a system can also broaden the spectrum of biocidal control: alternate feeding of two or more actives can have the same outcome as blending of the actives for simultaneous feeding.

Control of biofilms with biocides

It is well recognized in the industry that biofilms, or sessile microbial populations represent the group responsible for biofouling. Unfortunately, this is the group that is most difficult to control. Extracellular polymeric substances (EPS) contribute to the integrity of the biofilm and act as a physical barrier hindering biocides from reaching the living cell. Biofilm polymers can also consume oxidizers and some non-oxidizers before they reach and destroy microorganisms. As a result, control of biofilms requires dosages many times greater than required to control planktonic organisms. In practice, rapid biofilm regrowth and proliferation follow any inadequate control treatment regime. Even mechanical cleaning does not remove all traces of the biofilm. Previously fouled and cleaned or treated surfaces are more rapidly colonized than new surfaces. Residual biofilm materials promote colonization and reduce the lag time before significant biofouling reappears. Earlier published studies evaluated the comparative efficacy of several biocides (Haack et al., 1988; Grab and Rossmore, 1992; Brozel and Kloete, 1991). However, most of these studies were conducted on planktonic microorganisms or in field trials, and their results varied significantly due to variations in testing conditions. Author has developed an on-line real-time non-destructive continuous-flow system for biocide testing on industrial biofilms (Ludensky, 1998). This laboratory system is capable of monitoring changes in growth, accumulation and respiratory activity of biofilms in response to biocidal treatment. The system incorporates a fouling monitor for continuous measuring of the rate of biofilm accumulation (heat transfer resistance or HTR), a sensor for monitoring of microbial activity (oxygen meter for monitoring the rate of biofilm respiratory activity), and subsystems necessary for microbial life support and control of operation parameters (Figure 2). The sheathed *Sphaerotilus natans* (ATCC 15291), which is known to reside on heat exchanger surfaces in cooling water systems and papermaking machines, and is also associated with sewage treatment process upsets (such as the bulking of activated sludge), was selected for biofilm growth (Figure 3). HTR in this system corresponds to biofilm accumulation on the surfaces, while level of dissolved oxygen (DO) correlates with metabolic activity of biofilm. Examples of system operation and testing of oxidizing and non-oxidizing biocides are shown in Figures 4–9.

1. Typical biofilm growth. Figure 4 shows typical patterns of HTR and DO changes corresponding to attached growth of *S. natans*. The HTR curve showed significant exponential growth, while the DO level reduced sharply, according to strong respiration of the biofilm organisms in the presence of nutrients. Nutrient “addition and stop” events had a very strong impact on biofilm growth. The stop of nutrient addition was followed by a reduction in respiration rate. Reduced respiration initiated an increase in aqueous dissolved oxygen concentration due to saturation of the make-up water with oxygen. The theoretical exponential curve, calculated from an assumption that there is no biofilm in the system, and based on the half-life calculations, shows that about 5 hours would be required for the system to go from zero to almost 100% DO, and would stay at that level without biofilm. In the presence of biofilm, it took about 20 hours to reach a steady dissolved oxygen level about 85%, probably corresponding to endogenous respiration only. As soon as the nutrient pump was turned on again, DO level sharply decreased to the level they had before the nutrient was stopped. Somewhat different changes happened to HTR level: a sharp decrease in the first several hours was followed by a slow steady decrease, which continued until the nutrient was turned on. At this point, HTR began to increase, but at a pace slower than during initial growth phase in the first 72 hours. Biofilm, probably “takes time” to recover from nutrient starvation. Evidently, erosion of biofilm takes place without nutrient, followed by biofilm regrowth when nutrient is restored.
2. Effect of biocide. Figure 5 demonstrates the typical effect of an effective biocide on biofilm. In general, the behavior of the curve is similar to the nutrient stop: there are three portions on most curves. The first part usually shows the decrease in HTR level and increase of DO, probably, corresponding to erosion or removal of biofilm and a cessation or reduction in respiration. The second part of the curve could show a plateau in parameter value, corresponding to suppression of biofilm growth (some level of kill). The third part shows an increase in HTR and DO levels, corresponding to biofilm regrowth. It was anticipated, that depending upon the nature of the biocide, the mechanism of its activity and concentration, the shape of HTR and DO curves would indicate the biofilm response to the biocide. By comparing these curves to curves obtained previously, a definition of biocidal efficacy can be obtained.
3. Effect of oxidizing biocide. A slow-release oxidizing biocide (halohydantoin) containing methylethylhydantoin, bromine and chlorine (Dantobrom) was dosed at initial concentrations of 10 ppm as total Cl_2 for three hours every 24 hours (Figure 6). In case with oxidizing biocide suppression of biofilm accumulation was followed by fast regrowth. On the other hand, reduction in respiration response of biofilm to the second and the third

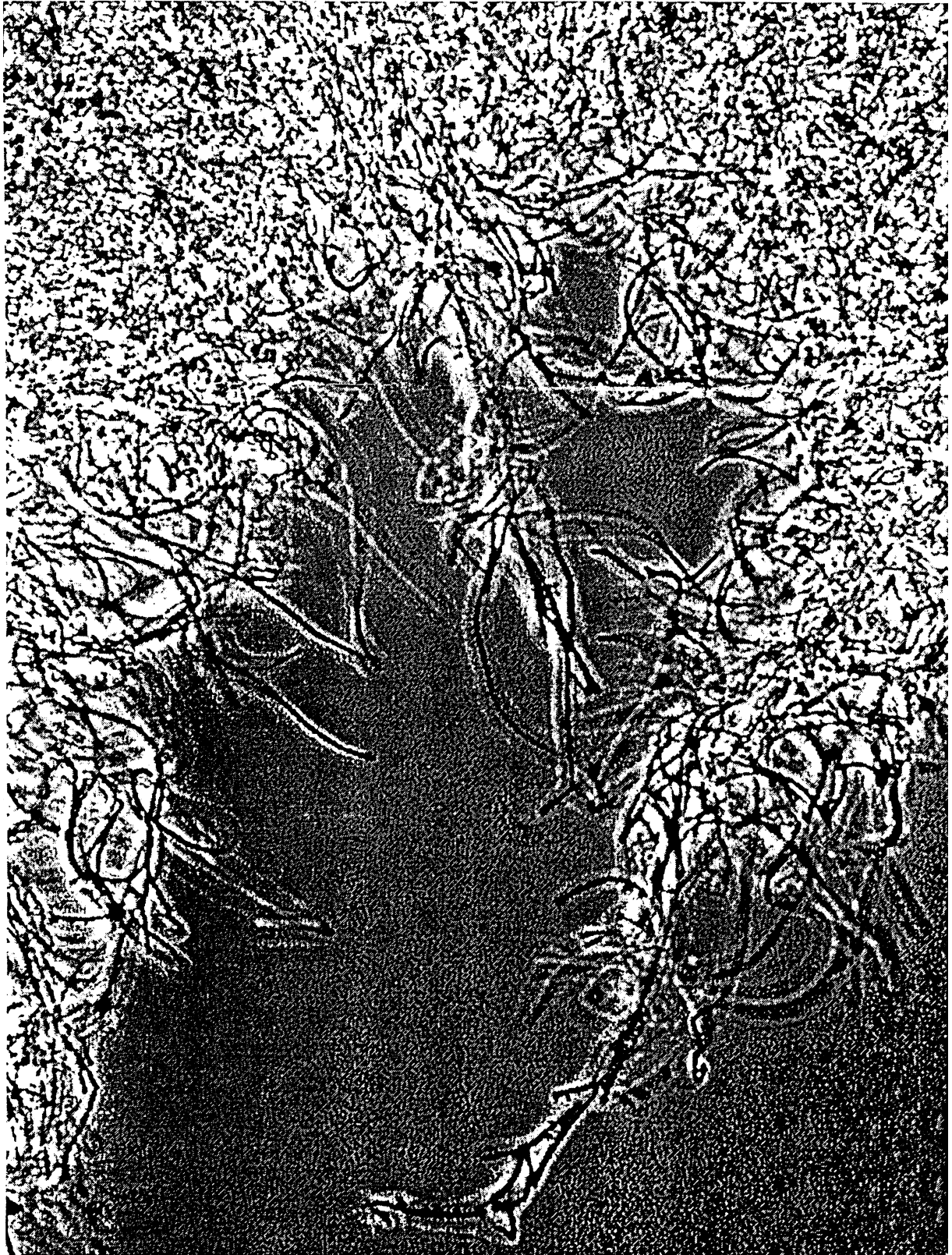


Figure 3 *Sphaerotilus natans* biofilm. Magnification 1000X.

treatments was less expressed than in experiment with non-oxidizing biocides. Probably, these experimental results reflect the difference in biocidal mechanisms between non-oxidizing and oxidizing biocides.

4. Effect of non-oxidizing biocide. The established biofilm was treated with non-oxidizing biocide (isothiazolone) at 4 ppm using the same treatment regime as in case with oxidizing biocide (Figure 7). This program showed diminishing efficacy of non-oxidizing biocide upon reapplication. Oxygen uptake increased with each treatment. This factor corresponded to fast recovery of the HTR level, especially after second and third

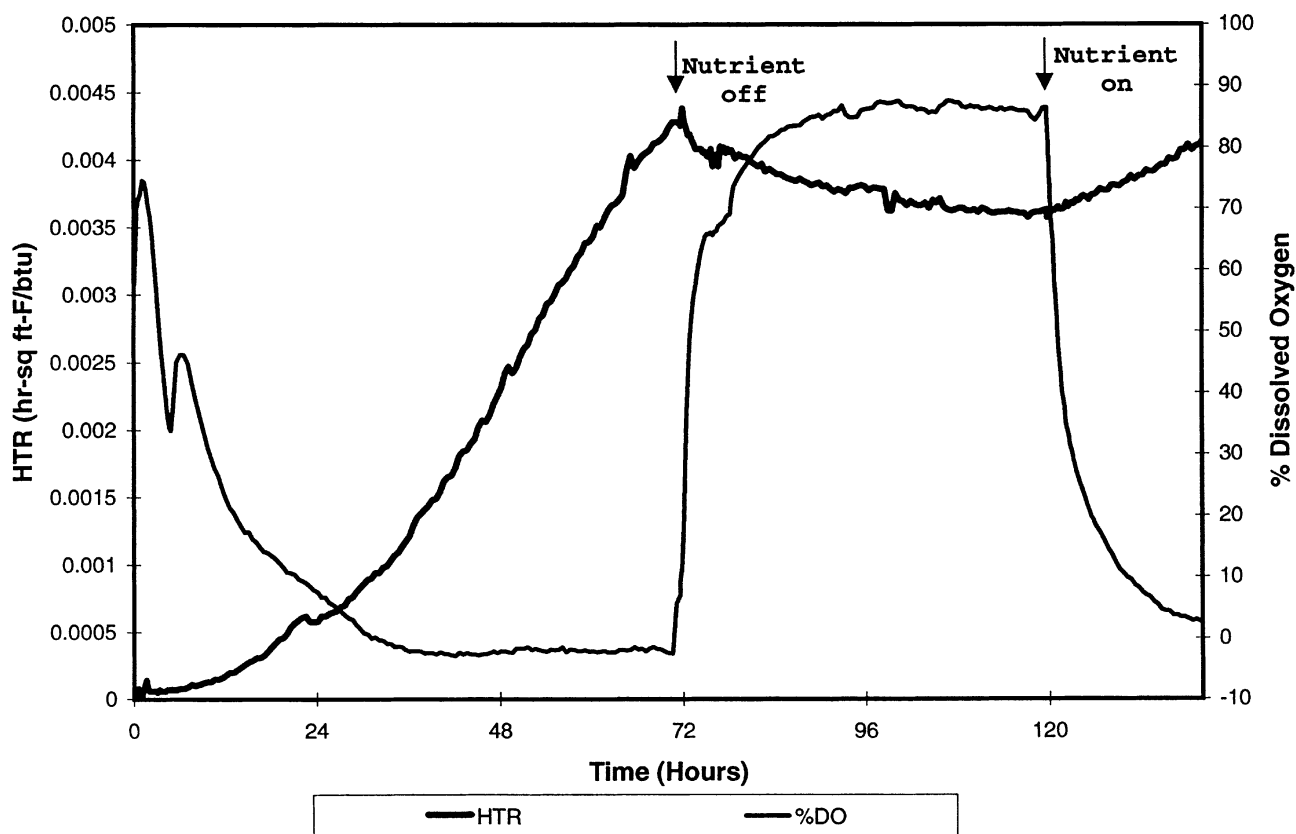


Figure 4 Typical biofilm growth of *Sphaerotilus natans* biofilm. Arrows correspond to the start and stop of nutrient addition.

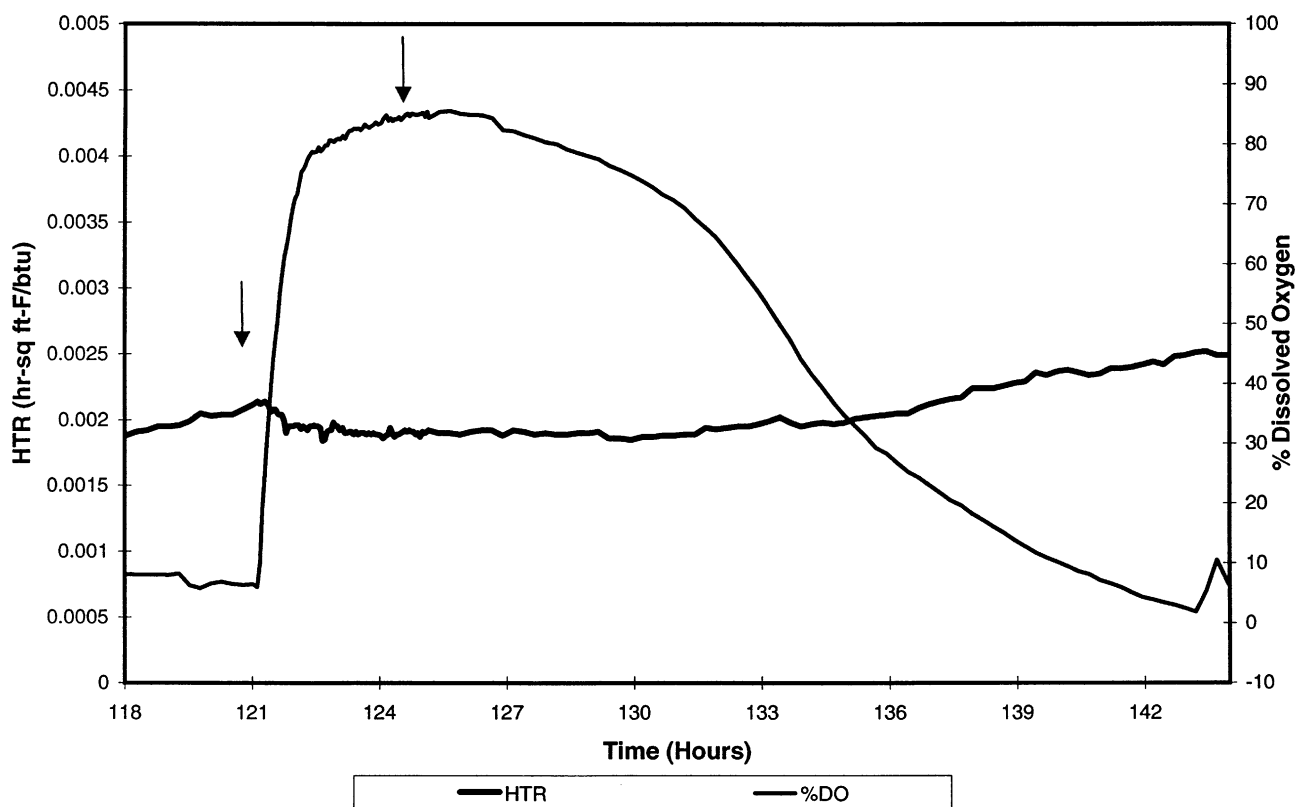


Figure 5 Effect of biocide on *Sphaerotilus natans* biofilm. Arrows correspond to the start and stop of biocide addition.

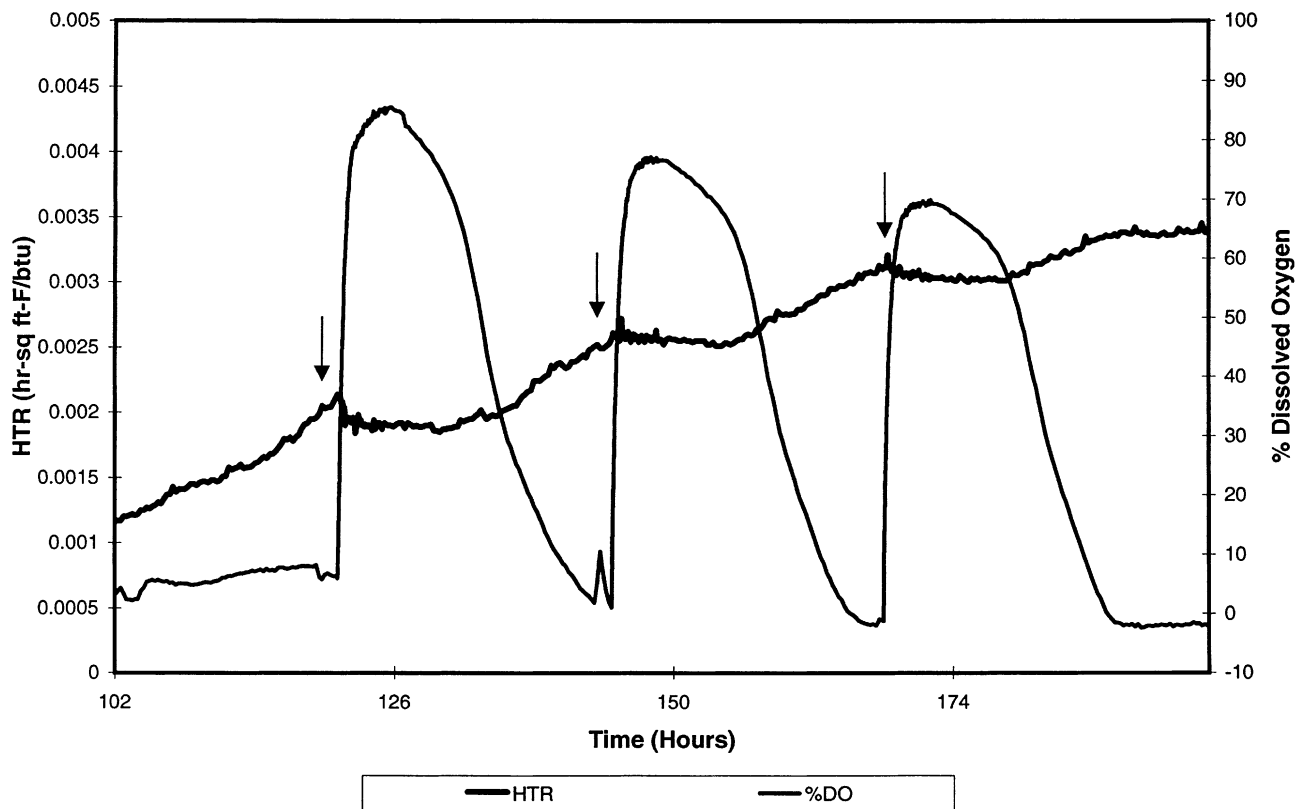


Figure 6 Biocidal efficacy of oxidizing biocide. Arrows correspond to addition of biocide.

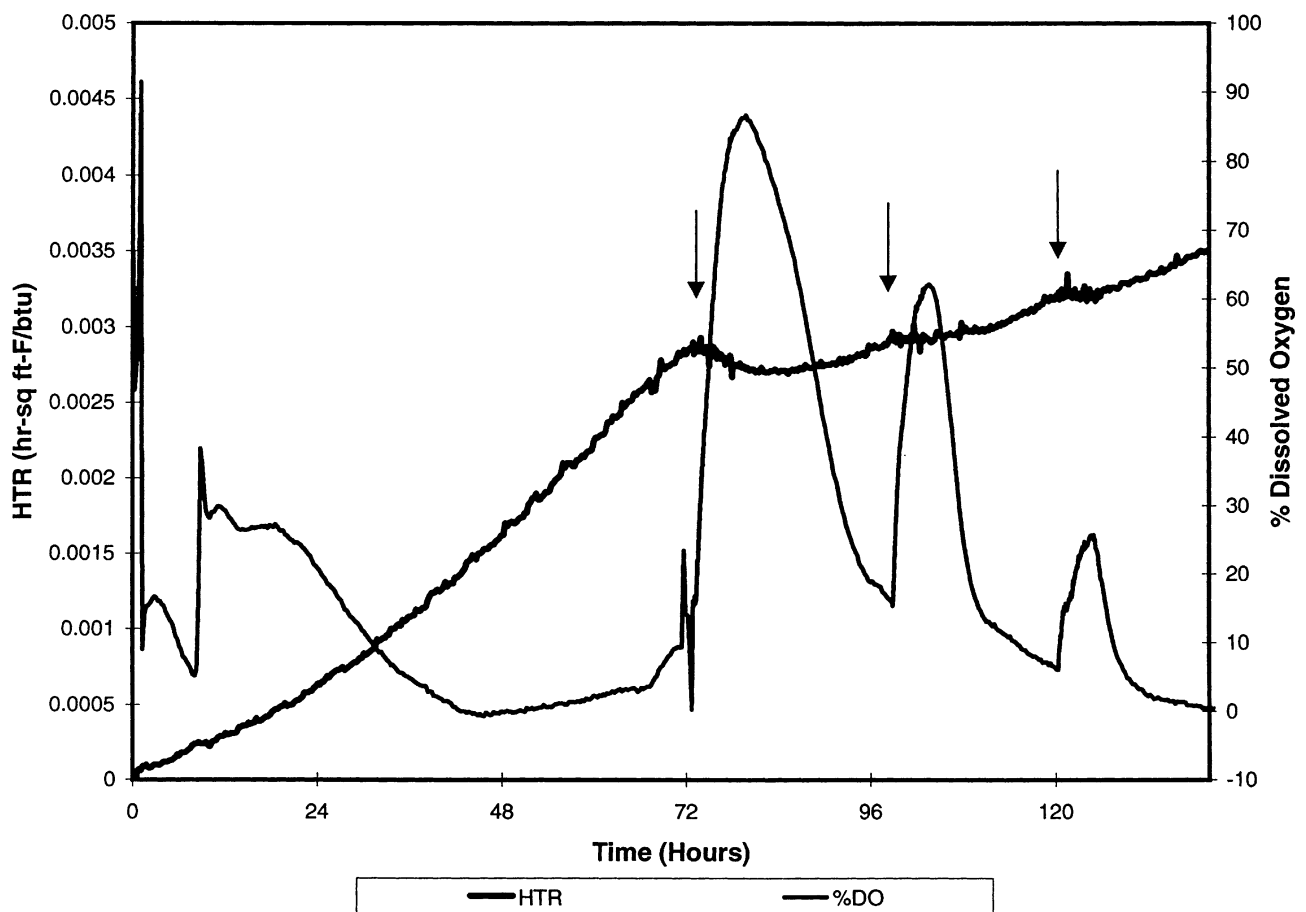


Figure 7 Biocidal efficacy of non-oxidizing biocide. Arrows correspond to addition of biocide.

treatments (recovery time for the first treatment was close to 24 hours, for the second and third treatments close to 5 hours).

5. Effect of combination of oxidizing and non-oxidizing biocide. The established biofilm was treated with a slug dose of isothiazolone at 4 ppm, which was followed in 1 hour by 3-hour continuous treatment with Dantobrom at 10 ppm (Figure 8). Results show that the recovery time for this two step treatment was around 36 hours, much longer than in case with oxidizing or non-oxidizing biocides when used alone. During 15 hours a complete suppression of biofilm respiration was observed, probably corresponding to a high level of biofilm cells kill. The regrowth observed in 36 hours was much smaller than in previous examples.
6. Effect of combination of non-oxidizing biocides. Experimental study shows that the combination of non-oxidizing biocides was more efficacious in biofilm control than biocides when used alone (Figure 9). Simultaneous use of isothiazolones (4 ppm) and quaternary ammonium compound (12 ppm) demonstrated better biofilm control than either isothiazolones or quat alone. This biocidal combination controlled biofilm (based on HTR data) for about 27, 30, and 32 hours after three consecutive treatments. The biofilm DO response was much more significant than any separately tested biocide and was similar to the test when isothiazolone was used together with oxidizing biocide. These laboratory results confirmed practical (field) evidence that combinations of biocides affect biofilms more effectively than biocides used alone.

Discussion

Due to the significant structural and functional complexity of biofilms, a complete explanation of the results obtained in this study is difficult. They can rather serve as a practical visual proof that biological activity of biocides depends on factors related to biocide properties, biofilm structure and water chemistry. Obtained results confirm practical conclusions that fouling problems found in cooling water systems can be traced directly to inadequate biological control (Freedman, 1979). In general, results of these experiments reflect previously anecdotal evidence of significant resistance of biofilms. For example, fast biofilm regrowth was observed for all biocides and combinations in our experiments. The changes in limiting nutrient and growth rate in the deeper layers of the biofilm structure following each biocidal treatment could be important reasons for the observed phenomenon of rapid biofilm recovery. The fact of diminishing efficacy of non-oxidizing biocides upon reapplication has been established for several non-oxidizing biocides (quats, glutaraldehyde, and isothiazolone). This effect is considered to be an example of multiple physiological resistance of biofilms to biocides. It has been demonstrated (Ludensky et al., 1998) that physiological resistance of biofilm to oxidizing biocides is less pronounced than for non-oxidizing biocides. Therefore, combination of both oxidizing and non-oxidizing biocides in one treatment, probably, diminishes physiological resistance of biofilm due to the dual mechanism of action.

We can only hypothesize that there are multiple reasons for recorded biofilm responses to biocides. It is recognized that biocide efficacy depends on several factors including transportation, adsorption, diffusion, penetration, and interaction at the target site. The differences in biocide efficacy also depend on the mode of action, chemical constitution and chemico-physical properties of biocide as well as on the chemistry of the medium and conditions of biocide application. Depending on the nature of a biocide, any of these parameters could be of major importance (Brown and Gilbert, 1993; Brown and Gilbert, 1995).

5.2.6 Environmental considerations

Biocide registration. See also Chapter 4

Prior to 1970 it was relatively easy to introduce a new product into the water treatment marketplace in the United States and other countries. As a result of legislation passed in the 1970s and 1980s, biocide manufacturers are required to meet stricter criteria in order to manufacture, ship and sell their product. The main EPA law for the registration of all pesticides, including antimicrobials is the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) that was first passed in 1947. In 1996 the Food Quality Protection Act (FQPA) was enacted, which establishes goals to protect the public, especially children, from exposure to harmful pesticides in food. The law also required EPA to treat antimicrobial pesticides separately and to streamline the registration process for nonfood antimicrobial pesticides. In 1998 the Antimicrobial regulation technical corrections Act (ARTCA) transferred authority over a number of pesticide residues to FDA. As a result, both EPA and FDA regulate cooling water biocides. Details of regulation of antimicrobial pesticides in the United States have been recently published (Sanders, 2001). Regulation of cooling water biocides in Europe and Pacific Rim are very region-specific, and can be found elsewhere. Since compliance with numerous government regulations is required, bringing a new biocide to the market is very complex. More testing is required and additional time and money must be allocated to meet new standards.

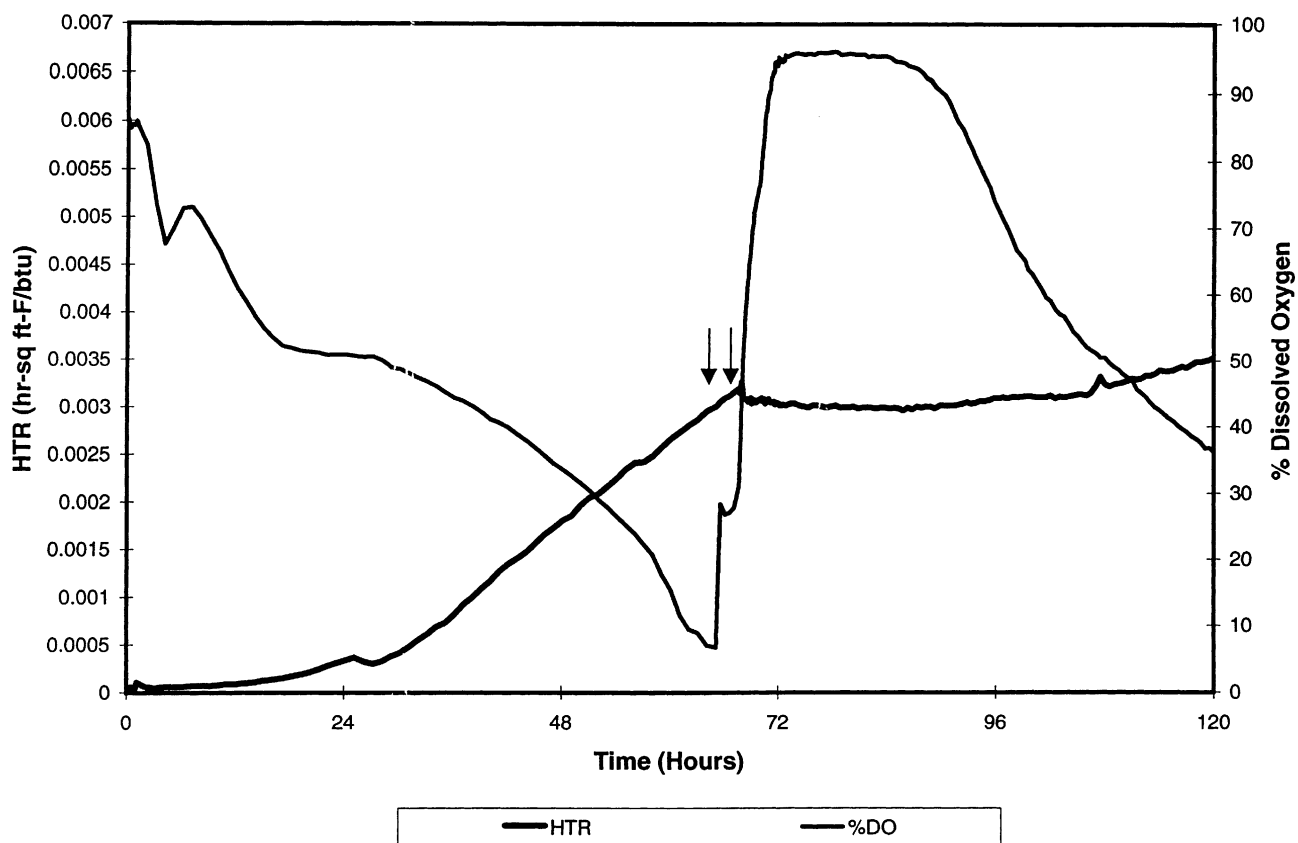


Figure 8 Biocidal efficacy of oxidizing biocide and non-oxidizing biocide. Arrows correspond to addition of biocide.

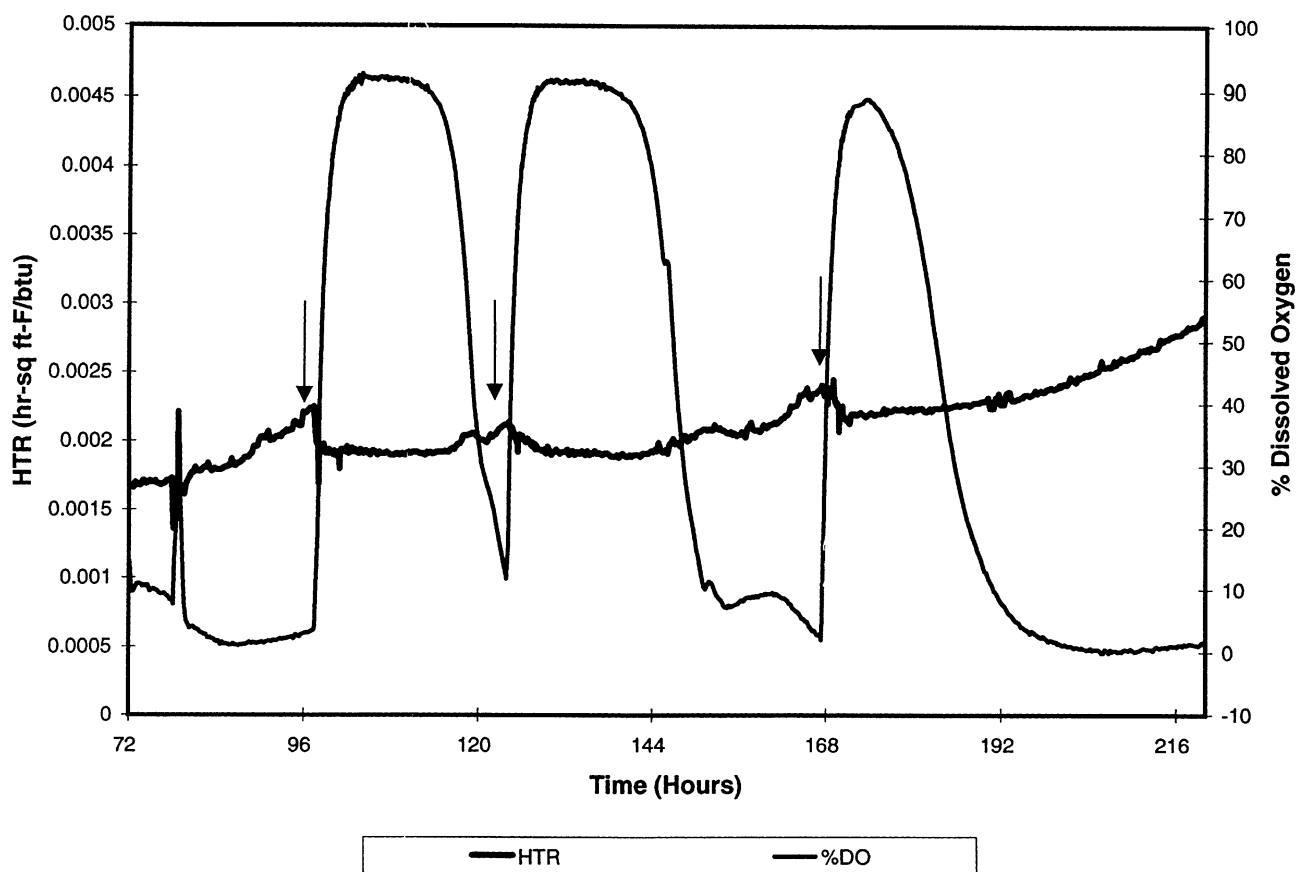


Figure 9 Biocidal efficacy of two non-oxidizing biocides. Arrows correspond to addition of biocide.

Discharge limitations

Environmental discharge and disposal considerations constitute an important factor, which determines the choice of microbicides. Disposal problems caused by toxicity have limited the use of certain biocides in many areas. In other situations, the microbicide chosen must be easily detoxified before cooling water discharge reaches receiving water bodies. Regulatory agencies historically have favored chlorine or chlorine compounds over bromine or non-oxidizing biocides. However, each state or regional regulatory agency tends to have its own biases that must be considered. For example, many areas of the United States have chlorine discharge limit of 0.2 mg/l of total residual chlorine for 2 hours per day. When biocides must be used at levels or duration that exceed the plant discharge limits, neutralization must be employed. Chemicals commonly used are strong reducing agents such as sulfur dioxide, sodium sulfate, and iron salts, as well as clay (for cationic biocides). Very often current discharge restrictions under which plant has to operate define selection of biocidal program.

5.2.7 Current trends in biocide development and applications

The regulatory changes that have taken place have substantially increased the cost of developing new biocides. Nowadays, biocide producers need to anticipate and address regulatory pressures, environmental safety concerns, as well as common sense perceptions of the end users in order to stay competitive in the current markets. Creative product handling strategies, new product forms and delivery options that minimize waste and on-site handling are in demand to differentiate biocidal products. The major service companies continue to move service and product supply programs toward high technology, including proprietary microprocessor control, new chemical delivery systems, novel on-line monitoring systems. As aging wooden cooling towers are replaced with non-rotting materials, the demand for fungicides is decreasing. The majority of cooling towers are now operated at pH > 8.0. This has created a growth opportunity for biocides that perform well at alkaline pH, and, consequently has eroded opportunities for products that function best at pH < 7.0.

No revolutionary new biocidal products for cooling water systems are expected in the near future. The continuing emphasis is on the use of known biocidal products with benign environmental profile. New combinations will make maximum use of available biocide functional capabilities.

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5.3 Recreational water treatment biocides

M.J. UNHOCH and R.D. VORE

5.3.1 Introduction

“Recreational water” is a term used to refer to a variety of water types: swimming pools, spas (hot tubs are a subset of spas with a wooden shell), water amusement parks, ocean beaches, rivers, and lakes. Swimming pools and spas are the only recreational waters that are routinely treated with biocides. Water parks may or may not apply biocides in their water handling systems. From a biocide supplier view, and the definition used in this chapter, “recreational water” will refer exclusively to swimming pools and spas.

By far the largest number of swimming pools and spas in the world reside in the United States. The latest estimates show that there are 7.8 million pools and 7.7 million spas in the United States. Approximately 95% of the US pools and spas are residential. The average US residential pool is 17,000 gallons whereas the average residential spa is 300 gallons.

There is no international glossary of terms for recreational water. The terms disinfectant, sanitizer, and shock lack internationally recognized definitions. Even in the highly regulated US market, governmental regulatory agencies, biocide manufacturers, distributors and end users frequently use slightly different terms. One example of differing definitions is of “sanitizer” and “disinfectant”. A disinfectant is defined in the US by the Environmental Protection Agency (EPA) as any chemical biocide passing the “AOAC Disinfectants (water) for Swimming Pool” rate of kill test. Those chemicals that do not pass the AOAC rate of kill test but that do pass the EPA field efficacy requirements are classified as “sanitizers”. Thus the term “sanitizer” is a slightly less efficacious treatment under laboratory conditions but is the functional equivalent of a “disinfectant” under use conditions. The National Pool & Spa Institute (NSPI), the largest trade organization of pool and spa manufacturers and dealers in the United States, has adopted the term “sanitizer” for all biocides registered by the US EPA for the control of pathogenic bacteria in pools and spas. In Europe the term “sanitizer” and “disinfectant” are juxtaposed in level of efficacy. The term “shock” is also poorly defined and may or may not imply biocidal treatment, depending upon context. As this chapter goes to press the US EPA is collaborating with the NSPI and numerous biocide suppliers through the offices of the American Chemistry Council to develop a more rational approach to the use of the word “shock” in the recreational water sector. The outcome of those efforts remains uncertain at this time. Lacking a recognized international glossary, the terminology used in this chapter will be consistent with that used by the NSPI (NSPI, 2002A).

In recreational water many biocides have been used either separately or in combination with other supplementary chemicals such as: biocides to control algae, uv stabilizers, shock for controlling accumulation of organics, or water balance adjustment chemicals for maintaining pH, total alkalinity and calcium hardness. The ideal recreational water biocide should possess the following properties:

- Effective against both pathogens and nuisance microorganisms
- Low order of toxicity or preferably non-toxic to humans, animals and plant life
- Safe to handle
- Does not degrade into or react with other pool or spa chemicals to form harmful disinfection by-products
- Stable shelf life at storage temperatures for a minimum of one year
- Water soluble at concentrations necessary to control the micro-organisms
- Efficacious in presence of and stable to organic impurities from bathers and environment, high and low water temperature, pH variations, differing water qualities and sunlight
- Should not impart color, odor or taste to the water
- Able to be monitored with a simple point of use test kit
- Should not discolor or damage pool and spa surfaces and equipment.

Swimming pools and spas are structures that hold water and are equipped with a circulatory system and filter. The circulatory system of pools includes the piping, pump(s), and filter(s). Water enters the circulatory system through skimmers and main drains. Skimmers vary in design but all designs serve to capture surface debris as well as water from the upper portion of the water column. Main drains are located in the deep end(s) and ensure circulation of the entire water column. After water enters in the skimmer(s) and main drain(s) it is directed through the pump and into the filter. After filtration the water is returned to the main pool through one or more return jets. Filter types used include diatomaceous earth, cartridge and sand beds. Most public pools employ sand filters while residential pools are nearly equally split among the three types. Residential spas commonly use cartridge filters. There are significant differences between pools and spas that are a reflection of physical parameter variations. Spas are smaller bodies of water where the entire water column is passed the filter in

10 minutes or less. The turnover rate in pools is usually at least 24 hours due to incomplete mixing. User exposure times in spas are usually 15 minutes. This is in contrast to pools where swimmers, especially children and adolescents often have use periods of greater than two hours. Spas also have higher water temperatures 100–104°F versus 75–85°F for pools, aeration (high-pressure air and water injected through jets) and higher ratio of bather waste to water volume. The differences in these physical parameters result in entirely different biological problems between pools and spas (Vore and Unhoch, 1996).

Why is it essential to treat recreational water with biocides? Bathers introduce bacteria from their skin, groin, urine, feces, and vomitus. When bathers introduce pathogens the water serves as a direct conduit for person-to-person disease transmission. Pathogens spread in this manner include: *E. coli* O157:H7, *Staphylococcus aureus*, *Cryptosporidium parvum*, and *Giardia intestinalis* (Lee et al., 2002; Barwick et al., 2000). Bacteria, fungi and algae are also introduced from the soil, leaves, fill water, and lower animals (birds, reptiles, amphibians, and insects). Virtually any microorganism found in the soil may be isolated from recreational water. Since recreational water provides near optimal conditions for growth (abundant water, mesophilic temperatures, neutral pH, and a variety of nutrients from bather waste and the environment) a biofilm consortium thrives within the circulatory systems of pools and spas. The density of bacteria in the biofilm on the surfaces of piping is normally 10×4 to 10×6 CFU/cm². Additionally, the filter media provides a large surface area for the attachment and proliferation of microbes. The flow of water through the piping provides a supply of oxygen and nutrients. The filter media normally contains 10×5 to 10×7 CFU/g of bacteria and fungi. Using conservative estimates, even small 100 pound residential sand filters harbor $>10 \times 9$ microorganisms. Industry literature describes the filter as a device for mechanical removal of particulates $>10 \mu\text{m}$. Considering the heavy microbial mass present, it is more appropriate to consider the filter as a fixed-bed bioreactor. When the appropriate concentration of the sanitizer is maintained the water column seldom exceeds 200 CFU/100ml of heterotrophic bacteria. If an inadequate sanitizer residual is maintained for several days or the circulatory system is malfunctioning the biofilm consortium greatly expands and develops into an infestation. During infestations *Pseudomonas aeruginosa*, *Legionella pneumophila* and expanded populations of normally non-pathogenic bacteria pose human health threats (Vore et al., 1998).

Recreational water is not sterile and cannot be sterilized even under ideal conditions. Biocidal treatments for recreational water are designed to manage the levels of microorganisms in the water and on the visible surfaces. Biocidal treatments for recreational water can be divided in the three categories: routine sanitation, preventative algicidal treatments, and remedial treatments. Sanitizing treatments are designed to yield rapid rates of kill (<10 minutes and in some cases <30 seconds) and prevent the person-to-person spread of infections. Preventative algicidal treatments inhibit the development of visible algal growth. Remedial treatments may be either for reduction of bacteria or algal masses.

Reliable information on recreational water biocides is generally lacking. In the collective 30 plus years the authors have worked in this industry we have concluded the recreational water industry is rife with mis-branded products that make outlandish and often fraudulent claims. We have attempted to rely on the most reliable information on specific products available, much of that acquired through personal experience. The National Spa and Pool Institute (NSPI) has published some of the more reliable product specific information. The NSPI published information bulletins are produced by the collaboration of scientists from Arch Chemicals, Avecia Inc., Biolab, Inc., DuPont, Church & Dwight, LaMotte Company, Occidental Chemical Corp., PPG Industries, Watkins Manufacturing Inc., Zodiac, and numerous private consultants. Information bulletins from the NSPI are the trade equivalent of peer-reviewed journal articles. The reader is advised to investigate trade journal and individual product performance claims thoroughly.

5.3.2 Sanitizers

A variety of biocides have been promoted as sanitizers in recreational waters. They include biocides that provide free chlorine and/or bromine, iodine, polyhexamethylene biguanide, quaternary amines, copper sulfate, silver, ozone, ultraviolet light, hydrogen peroxide, and combinations of these. Although these are all biocidal, not all of these treatments provide a sufficiently rapid kill to maintain the sanitary state of the water. There are no global performance criteria for sanitizers. US standard, which is closely mirrored by the Canadian standard, is recognized as being sufficiently high to protect bathers from microbial health threats as well as from unwarranted chemical hazards. This chapter will focus only on those chemicals that have been granted registration as disinfectants or sanitizers by the US EPA. Several biocides (ozone, chlorine dioxide, silver) may serve as supplementary biocidal treatments for use in combination with a primary sanitizer. These will be addressed separately below.

Primary sanitizers are those chemicals that can maintain the sanitary state of the water by their own activity under prescribed field test conditions (EPA, 1979). Table 1 lists all biocides that been registered by the EPA as primary sanitizers as well as their primary use pattern, use concentration, and the advantages and disadvantages

Table 1 Summary of use patterns and characteristics of primary sanitizers

Biocide	Primary application	Classification	Use concentration	Advantages	Disadvantages
Sodium Hypochlorite [II, 21.2.2a.]	Pool	Sanitizer	1.0–3.0 ppm as HOCl	Completely water soluble	Not shelf-life stable Raises pH and increases acid demand
Calcium Hypochlorite [II, 21.2.2b.]	Pool	Sanitizer/Shock	1.0–3.0 ppm as HOCl	Shelf-life stable Easy to handle Completely water soluble	Raises pH and increases acid demand Significantly raises calcium hardness
Lithium Hypochlorite	Pool	Shock	1.0–3.0 ppm as HOCl	Completely water soluble Shelf-life stable Easy to handle	Highest cost chlorine shock
Sodium Dichloro-s-triazinetriene [II, 21.2.6.]	Pool/Spa	Sanitizer/Shock	1.0–3.0 ppm as HOCl	Shelf-life stable Completely water soluble Little effect on pH and acid demand Easy to handle	Most expensive stabilized chlorine Significantly raises cyanuric acid
Trichloro-s-triazine-trione [II, 21.2.7.]	Pool	Sanitizer	1.0–3.0 ppm as HOCl	Slow dissolving Shelf-life stable Highest available chlorine content	Lowers pH, total alkalinity and increases base demand Raises cyanuric acid
Sodium bromide + Activator [II, 21.2.3a.]	Spa	Sanitizer	2.0–4.0 ppm (some products vary slightly)	Easy to handle Effective over broad pH range Microbial activity unaffected in presence of organics	Not stable to sunlight Lower pH Periodic shock treatment required
1,3-bromo-chloro-dimethylhydantoin (BCDMH) [II, 21.2.11.] 1,3-dibromo-dimethylhydantoin (DBDMH) Polyhexamethylene Biguanide [II, 18.3.3.]	Pool/Spa	Sanitizer	2.0–4.0 ppm (some products vary slightly)	Easy to handle Effective over broad pH range Microbial activity unaffected in presence of organics	Not stable to sunlight Lower pH Periodic shock treatment required
	Pool/Spa	Sanitizer/Algistat	6.0–10.0 ppm as active	Easy to handle Completely water soluble Does not effect water balance Stable to sunlight Effective over wide pH range Shelf-life stable Effective in presence of high organic load	Requires a separate oxidizer Increases maintenance of filter More expensive than chlorine products
Silver	Pool/Spa	Sanitizer	1.0 ppm as product	Minimal handling hazards.	Staining of surfaces endemic Separate oxidizer required.

for each biocide. The concentrations listed in Table 1 are as per their EPA approved directions for use. The NSPI chemical operating parameters specify up to 10 ppm chlorine (NSPI, 2002B). The NSPI parameters were written to allow greater operator flexibility in large public facilities where halogen demand can display sudden abrupt increases. The NSPI parameters lack legal standing and should be followed with caution.

Primary sanitizers are designed to control bacterial growth, but may not be as effective against algae or the nuisance fungi. Conversely, biocides that control the non-public health threat nuisance organisms seldom are efficacious against bacteria. Therefore, two or more biocides (sanitizer and algicide) are routinely employed to maintain a pool or spa free from both pathogens and nuisance organisms.

5.3.2.1 Halogens and hypohalogenites and halogen release compounds [II, 21.2.]*

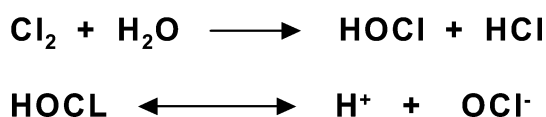
The discovery, investigation of biocidal properties, and the development of practical treatments using halogens has been documented by others (Dychdala, 1991; Hurst, 1991; White, 1972).

Halogens, hypohalogenites and halogen-release compounds can include all of the elements and compounds containing the elements listed in Group VII A of the Periodic Table. Chlorine and bromine are the only halogens presently used in swimming pools and spas. Both chlorine and bromine are terms used by the industry generically to represent any compound that will produce hypochlorous or hypobromous acid.

*see Part Two – Microbicide Data

There are two classes of chlorine commonly used in swimming pools referred to as unstabilized and stabilized. To protect hypochlorous acid from UV degradation cyanuric acid is added to the water. Whether the cyanuric acid is supplied endogenously by the product (stabilized) or whether the cyanuric acid must be added separately (unstabilized) distinguishes the classes. Unstabilized chlorine compounds are chlorine gas, sodium hypochlorite, calcium hypochlorite and lithium hypochlorite. Stabilized chlorine compounds are chlorinated forms of cyanuric acids, sodium dichloro-s-triazinetriene (dichlor) and trichloro-s-triazinetriene (trichlor). It is important to never mix stabilized and unstabilized chlorine products because they are not compatible and can be combustible when mixed as concentrates.

The oxidation potential of halogens is the result of their strong affinity to electrons. The introduction of any of the unstabilized or stabilized forms of chlorine produce hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) when dissolved in water. The total amount of both species is referred to as free available chlorine. Hypochlorous acid is the primary chemical responsible for sanitation. The hypochlorous acid and hypochlorite ion equilibrium is illustrated by the following equation:



Reaction of chlorine with water to form hypochlorous acid and dissociation of hypochlorous acid into hypochlorite ion

The maintenance of proper sanitation using chlorine-based biocides is based on understanding four basic reactions of hypochlorous acid: the dissociation equilibrium, the impact of ultraviolet light, the reaction of hypochlorous acid with ammonia and nitrogenous organic substances, and the reaction with bacteria and inorganic substances. Practical considerations of these reactions are detailed in all pool operator training manuals, including the most recent edition from the NSPI (NSPI, 2001). Detailed chemistry explanations may be found in the Handbook of Chlorination (White, 1972). The 1972 edition contains a chapter on pools, which has not been included in subsequent editions.

–Impact of pH on efficacy. Maintenance of the pH is quite important when relying on hypochlorous acid sanitizer systems. The operating range of pools and spas is 7.2 to 7.8 and is determined by bather comfort, especially the mucous membranes of the eyes. The pKa of the hypochlorous acid: hypochlorite dissociation is at a pH of 7.5. As pH rises above 7.5 the equilibrium reaction favors the hypochlorite (OCl^-) ion. This is not desirable since hypochlorite is 100 fold less efficacious than hypochlorous acid. The situation is exacerbated in spas, which due to air injection and the carbonate-bicarbonate buffering system used in recreational water, perpetually drift toward a pH equilibrium of 8.2. The total amount of hypochlorous acid and hypochlorite ion is referred to as free available chlorine (FAC). The EPA approved labels require 1.0 to 3.0 FAC be maintained. (Note: The EPA is currently increasing this to read 1.0 to 4.0 ppm. This will allow recreational water chlorinating products to be consistent with potable water chlorinating compounds.) Table 2 shows how subtle pH changes impact the hypochlorous-hypochlorite equilibrium.

This table clearly illustrates the importance of maintaining the pH of the pool when utilizing hypochlorous acid as the sanitizer. For example, at a pH of 7.2, the HOCl present in the water is 80.9%, whereas at a pH of 8.2, the HOCl is only present at 30%.

–Impact of sunlight on stability. Sunlight, or more specifically ultraviolet light, rapidly reduces hypochlorous acid to non-biocidal chloride salt. On a sunny day nearly 100% of hypochlorous acid is destroyed in 2 hours (NSPI, 1995). Cyanuric acid is used in many recreational water chlorine systems as a UV stabilizer. Many US public health codes limit the cyanuric acid level to an upper limit of 100 mg/L. The present NSPI chemical

Table 2 The effect of pH on availability of hypochlorous acid concentrations (at 25°C) (White, 1999)

pH	Percent HOCl
6.5	91.60
7.0	77.53
7.5	52.18
8.0	25.65
8.5	12.07
9.0	3.33

operating parameters state that 30 to 50 ppm cyanuric acid is ideal and that 150 ppm is the maximum permissible concentration (NSPI, 2002). When stabilized chlorine products are utilized the concentration of cyanuric acid increases over time. When this occurs the only effective option is to partially drain the water and refill with fresh potable water. It is difficult to compare the chlorine demand of outdoor with indoor pools but indoor pools generally have a lower chlorine demand. It is difficult to compare two such pools due to complicating factors including bather usage patterns, but it is believed that much of the difference is due to difference in loss of chlorine to UV, even when cyanuric acid is present in both pools.

While the sunlight-stabilizing effect of cyanuric acid on hypochlorous acid has been clearly shown, there is no general agreement about whether cyanuric acid significantly reduces the efficacy of FAC as an oxidizer or bactericide.

–*Reactions with ammonia and organic compounds.* Hypochlorous acid reacts rapidly with bacteria. Up to 90% of the hypochlorous acid consumed in recreational is consumed in oxidizing organic matter. When ammonia, urea or other nitrogenous compounds are present in the chlorinated water the hypochlorous acid reacts with the nitrogen forming N-chloro compounds. The reaction of ammonia with chlorine in water forms monochloramine (NH_2Cl), dichloramine (NHCl_2) and trichloramine or nitrogen trichloride (NCl_3), depending on the concentration of the reactants. Hypochlorous acids reacts with peptides and proteins to form similar related compounds. These are generically referred to as chloramines. Simple chloramines are bactericides, but the rate of kill is slow. Monochloramine is increasingly being employed in potable water distribution systems because of its persistence. Monochloramine is not an effective sanitizer for recreation water because the rate of kill is 60 to 100 times slower than that of hypochlorous acid. Chloramines are objectionable because they are odorous and cause burning eyes. The total chlorine (TC) is the total amount of free available (FAC) and combined chlorine (CAC) in the water.

–*Breakpoint chlorination.* Breakpoint chlorination is a historical concept where combined chlorine is re-oxidized to hypochlorous acid by the addition of an excess concentration of hypochlorous acid. These are collectively referred to as combined chlorine or combined available (CAC) under the assumption that the chlorine can be re-liberated. The model used for nearly all literature cites the interaction between hypochlorous acid and ammonia. In recreational water the amount of hypochlorous acid used for a breakpoint treatment is normally ten times the concentration of the combined chlorine. However, the reaction between hypochlorous acid and more complex nitrogen compounds is not fully reversible. White (1986) showed that breakpoint water containing a mixture of combined chlorine from organic and simple ammonia failed to display the classic dip of the breakpoint reaction. These waters displayed a plateau concentration below which no further reduction in combined chlorine occurred. The nitrogen compounds in recreational water are introduced in bather waste and from the environment and contain mostly amino acids, peptides, and proteins with little free ammonia. Practical experience has shown that this method will reduce, but not eliminate, the combined chlorine. If repeated breakpoint treatments fail to reduce the combined chlorine to the target level (0.02 to 0.05 ppm CAC) alternate treatments such as oxidation with a potassium monopersulfate or partial water replacement to dilute the chloramines must be used.

Other by-products associated with chlorine/hypochlorite disinfection include trihalomethanes (THM's), haloacetic acids, haloacetanitriles, haloketones, chloral hydrate (trichloroacetaldehyde), chloropicrin (trichloro-nitromethane), cyanogen chloride and chlorate (WHO, 2000).

–*Superchlorination.* Superchlorination is defined as, “The practice of adding a sufficient amount of a chlorinating compound to reduce cloudy water, slime formation, musty odors, algae and bacteria counts, and/or improve the ability to maintain sanitizer residuals” (NSPI, 2002B). The NSPI definition is consistent with the realization that superchlorination provides a dual function of significantly increasing the oxidation potential in the water as well as providing increased biocidal functionality through the elevated hypochlorous acid. Superchlorination practices have evolved after the realization that breakpoint chlorination is insufficient to manage the accumulation of organic material in a chlorine sanitized pool.

–*Neutralization.* Many health codes regulate bather entry to specified concentrations of hypochlorous acid in the water. This varies, but in the US bathers are generally not allowed in the water if chlorine levels are above 3.0 ppm. This is referred to as the “re-entry level”. After superchlorination it may be necessary to partially dechlorinate the water to re-entry levels. This is accomplished by the addition of a chlorine neutralizer (reducing agent), such as sodium sulfite, sodium thiosulfate or hydrogen peroxide.

–*Reaction with metallic ions.* Hypochlorous acid reacts rapidly with metal ions, especially Fe(II), and Mn(II), and is reduced to non-biocidal chloride (Cl^-). The metal catalysis of hypochlorous acid is the motivation for routine analysis of recreational for metal ion content (White, 1999).

–*Test methods.* There are a number of direct and indirect test methods used for the measurement of chlorine residual. The commonly used direct methods include, colorimetric and titration and the indirect method commonly used in commercial and some residential is oxidation-reduction potential.

–*Colorimetric-DPD (N,N'-diethyl phenylenediamine) method.* The most common, and the preferred method of measuring free and combined chlorine concentration is the N,N'-diethyl phenylenediamine (DPD) method. Chlorine-containing compounds (HOCl , OCl^- , NH_2Cl) react with the DPD indicator to form a pink color. Chloride ions do not react or interfere with the test. In a two-stage process free available chlorine (FAC) and total chlorine (TC) are determined separately. Combined available chlorine (CAC) is calculated from the equation:

$$\text{CAC} = \text{TC} - \text{FAC}$$

(NSPI, 2001). The determination of CAC is vital in maintaining the proper sanitary state as well as ensuring bather comfort. (See breakpoint and superchlorination above.)

–*Colorimetric-OTO (orthotolidine) method.* The Orthotolidine (OTO) method is based on the reaction of total chlorine species ($\text{FAC} + \text{CAC}$) with the colorless OTO indicator to produce a yellow-orange-red color. The intensity of the color is proportional to the concentration of the chlorine species present. The color is compared to previously prepared color standards. Chloride ion (Cl^-) will not react with the OTO indicator (NSPI, 2001). Because the OTO method cannot differentiate free from combined chlorine it is slowly being replaced by the DPD method. Many US local and state health jurisdictions no longer accept OTO as a monitoring method.

–*Test strips.* Test strip methodologies were originally developed for the medical industry for testing blood and urine. Miles Laboratories introduced the first dip-and read strips in the late 1950's (Morris and Sweazy, 2002). Test strips incorporate a solid reagent into a paper matrix. The reagents are activated by water contact. The resulting color intensity is compared to a chart to provide a level of total and free chlorine. Test strips are a preferred method for residential pools and spas and less technically sophisticated user because the color intensity is not dependent on water volume; this is in contrast to liquid reagent tests that are volume dependent. Many test strips currently available also include tests for water balance parameters (pH, total alkalinity and total hardness). Test strips are commonly used for monitoring chemical levels in both pools and spas. It is important to keep the unused strips dry because they are sensitive to moisture.

–*ORP (oxidation-reduction potential).* Oxidation-Reduction Potential (ORP) or Redox is the activity or strength of the oxidizer on the basis of their concentration. ORP measurements can be used to indirectly measure the concentration of several biocides including chlorine, bromine, ozone, and chlorine dioxide. In 1982 the German Standards Agency adopted 750 mV standard for public pools. The NSPI supports a 650 mV minimum reading where ORP is used to control chlorine. Typical ORP sensors are platinum/platinum probes. Output is measured in millivolts. It is becoming a common, but ill advised, practice to measure the concentration of chlorine by ORP alone. ORP readings must be adjusted for pH. The output of the probe is greatly impacted by the oxidation state of the platinum element. The NSPI recommends that where ORP probes used to control halogen concentration the chlorine should be manually measured at least daily (NSPI, 2002A). ORP cannot be used to control chlorine if multiple oxidizing compounds are present. This would be the case if a chlorine pool were oxidized with monopersulfate or chlorine dioxide.

5.3.2.1.1 Unstabilized chlorines. Chlorine gas, sodium hypochlorite, calcium hypochlorite, and lithium hypochlorite do not contain cyanuric acid as part of its makeup and are classified as unstabilized. However, they can be stabilized with the addition of 25 to 150 ppm of cyanuric acid directly to the pool.

5.3.2.1.1.1 Chlorine gas [II, 21.2.1]. Chlorine is a greenish-yellow, water-soluble gas with a pungent odor. It has the greatest amount of available chlorine of all chlorine products at 100% (by definition). Chlorine gas is extremely toxic and was used as a chemical weapon during World War I. Even at low concentrations chlorine gas is extremely irritating to the skin, eyes, nose, throat and lungs. Because of the hazards, the use of gaseous chlorine is restricted to commercial facility with specialized handling facilities and to residential pools in select locations. The toxicity of biocide mandates thorough training of the applicators. For residential use professional applicators that inject the gas directly into the pool from storage tanks maintained on the service truck administer the biocide. These applicators are known as “gas shooters”.

Chlorine gas will significantly lower pH upon each addition. Therefore, a buffer (sodium bicarbonate) or strong base (sodium carbonate) needs to be used with it (Nalepa, 1997).

5.3.2.1.1.2 *Sodium hypochlorite* [II, 21.2.2a]. Sodium hypochlorite is a clear, slightly yellow liquid aqueous. Household bleach has active concentrations between 5.25 and 6.00% whereas; concentrations of 5 to 15% are used in the industry. Most commercial applications use the more concentrated solutions. One gallon of sodium hypochlorite yields approximately the same quantity of active chlorine as one pound of chlorine gas.

Sodium hypochlorite increases the pH of the water. It also increases the total dissolved solids in the water over time. Solutions have a limited shelf life (about 6 months) and should not be stored in direct sunlight or areas capable of high temperatures. During extended or improper storage the concentration of active declines producing less desirable, but still usable product.

The use patterns of sodium hypochlorite are not uniform in the US. Use as a primary sanitizer is limited to regions with extended seasons (Florida, Arizona and California). In these regions sodium hypochlorite is distributed to retail outlets as bulk liquids. End users purchase the material in re-usable carboys.

Sodium hypochlorite is corrosive to eyes, skin and mucous membranes. The vapors are irritating. Use care during handling and application of material. Avoid contact with stabilized chlorine products, acids, metals, organics and strong reducing agents.

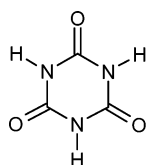
5.3.2.1.1.3 *Calcium hypochlorite* [II, 21.2.2b]. Calcium hypochlorite is a white granular compound with a chlorine-like odor. It is often sold in tablet, briquette and granular form. Calcium hypochlorite is 65–70% available chlorine. In addition to use as a primary sanitizer, it can be used to control algae and oxidize organic contaminants. Most calcium hypochlorite products are registered with the EPA as dual functional sanitizer and superchlorinators. The granular products are pre-dissolved in a bucket of water prior to adding to the pool or by direct addition. The compacted forms are used primarily to maintain a chlorine residual in the pool water and are either added to a floating chemical feeder or an in-line erosion feeder. Calcium hypochlorite will raise the hardness and pH of the water upon addition. The rise in pH is more significant upon use of superchlorination doses (NSPI, 1995).

5.3.2.1.1.4 *Lithium hypochlorite*. Lithium hypochlorite is a water soluble white granular solid. It has been used as a source of chlorine for several years. Lithium hypochlorite yields 35% available chlorine. It is more costly than the sanitizers previously listed. Lithium hypochlorite is readily soluble in water and does not affect the pH of the pool water as significantly as the other hypochlorites. In addition to use as a primary sanitizer, it can be used to control algae and oxidize organic contaminants. Its application is limited to superchlorination as a result of its higher price. A major advantage is that repeated usage does not increase the calcium ion concentration in the water column. Calcium hardness is a key factor in maintaining the correct saturation index (NSPI, 2001). High calcium concentrations can be problematic leading to scaling of pool and spa walls and equipment and cloudy water. In regions that have very hard water the use of lithium hypochlorite is preferred.

5.3.2.1.2 *Stabilized chlorine*. Stabilized chlorine products contain cyanuric acid as an integral portion of their makeup. Upon dissolution stabilized chlorine products liberate cyanuric acid into the water column and are therefore regarded as self-stabilizing. Stabilized chlorine products are either sodium dichloroisocyanurate (dichlor) or trichloroisocyanuric acid (trichlor). These are generically called the *isos*.

One difficulty with stabilized chlorine biocides is that cyanuric acid is constantly being added without much being removed by water carry-out, splash-out, or backwashing of sand filters, or through degradation. The results may be unacceptably high cyanuric acid and total dissolved solids (TDS) levels. When this condition occurs the levels must be reduced by dilution (draining a portion of the pool and replacing with fresh water).

5.3.2.1.2.1 *Cyanuric acid*. Cyanuric acid is an odorless, white granular substance generally referred to as a stabilizer, a conditioner, isocyanuric acid, or CYA. When cyanuric acid is used at prescribed 30 to 50 ppm concentration, the stability of free chlorine residuals is increased three to five fold in the presence of sunlight.

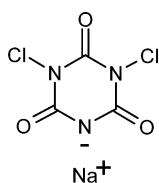


Cyanuric acid

NSPI's Chemical Operating Parameters list the maximum acceptable concentration of cyanuric acid as 150 ppm (NSPI, 2002A).

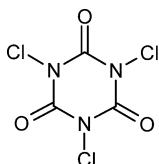
Cyanuric acid has the following properties: pH 4.5 (in a 1% solution), slow-dissolving, maximum solubility = 1,600 mg/L, not destroyed by any pool chemical, and is removed only by dilution (draining and replacing existing water with fresh), splash-out, and backwash.

5.3.2.1.2.2 Sodium dichloro-s-triazinetriene (sodium dichloroisocyanurate or dichlor) [II, 21.2.6]. Sodium dichloro-s-triazinetriene, Dichlor, has been used to sanitize pools and spas since the 1960s. Dichlor is usually sold as white granules in either anhydrous or dihydrate forms. The available chlorine for these chemicals are 63% and 56%, respectively. Several companies have recently introduced dichlor combined with other oxidizers. These blended products vary in their available chlorine content. Dichlor is readily soluble and is the only chlorine product with a nearly neutral pH (6.0, 1% solution @ 25°C) (NSPI, 1994). Dichlor is used in both pools and spas as a primary sanitizer. Dichlor will react with most other chemicals, especially unstabilized chlorines. Dichlor is sensitive to moisture.



Sodium Dichloro-s-triazinetriene

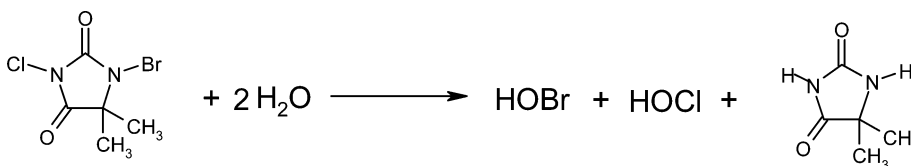
5.3.2.1.2.3 Trichloro-s-triazinetriene (trichlor, trichloroisocyanuric acid) [II, 21.2.7]. Trichloro-s-triazinetriene (Trichlor) contains approximately 90% available chlorine content. This is the second most concentrated form of chlorine, chlorine gas being 100% available chlorine. Trichlor is available as both a granular product and tablets. Tablets are primarily used to maintain chlorine residuals in swimming pools because of its very low water solubility (1.2% at 25°C). Trichlor tablets are added to the skimmer or used in conjunction with a floater or an in-line, erosion-type feeder. (Note: it is seldom permissible to feed any chemical through skimmers or in floating feeders in commercial settings due to the possibility of patron access to the concentrated product). Although it is convenient to use, trichlor is strongly acidic. If not monitored, the resultant low total alkalinity and high pH will cause corrosive damage to the pool surface and equipment. Trichlor granules are used as a treatment for black algae in plaster pools. Granular Trichlor should not be used in vinyl pools because it is slow dissolving and will bleach the liner. Trichlor will react with most other chemicals, especially unstabilized chlorines so they should never be mixed, and is sensitive to moisture (NSPI, 1995).



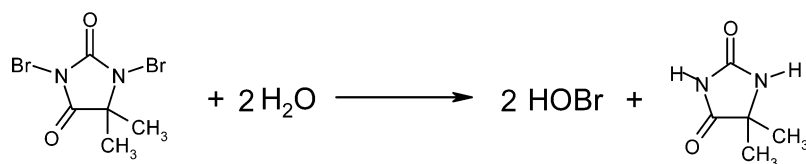
Trichloro-s-triazinetriene

5.3.2.1.3 Bromine. Bromine was introduced as a pool water sanitizer in 1947 in the form of elemental bromine. Due to potential hazards associated with handling liquid bromine, bromine sticks were introduced about 1958 (White, 1999).

Bromine sticks are composed of 1,3-bromo-chloro-dimethylhydantoin (BCDMH) [II, 21.2.11.]. 1,3-dibromo-dimethylhydantoin (DBDMH) was introduced in 2000. The BCDMH hydrolyzes to form both hypobromous and hypochlorous acids, whereas, the DBDMH only releases hypobromous acid [II, 21.2.3.] upon hydrolysis.



BCDMH release of hypochlorous and hypobromous acids



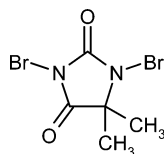
DBDMH release of hypobromous acid

Another bromine system used primarily in spas is a two-part system consisting of sodium bromide [II, 21.2.3a.] and an oxidizer. Whichever bromine product is used, the active biocide produced is hypobromous acid. The high cost of bromine and instability to sunlight makes its use for outdoor pools relatively uneconomical.

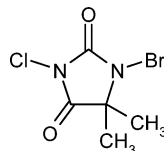
Bromine is not as effective as chlorine in combating algae and oxidizing organic matter in the water. It sometimes causes a green color in the pool and occasionally imparts a brown discoloration to pool walls. Where local authorities permit bromination, the normal range for total residual bromine varies between 2.0 and 4.0 ppm (NSPI, 2002A).

–*Sodium bromide.* Bromide ion is not a biocide. Bromide ion can be readily oxidized to produce the biocidal hypobromite ion or hypobromous acid. The oxidizer can be chlorine, ozone or potassium monopersulfate.

–*DBDMH and BCDMH.* These slow-dissolving tablets are used in either a floater or in-line erosion feeder. When bromine tablets are added to water, hypobromous acid (HOBr) is produced along with bromide ion (Br^-). The bromide ion is ineffective in pool sanitation. The bromide ion in a pool can be activated, or reactivated, to hypobromous acid using chlorine, ozone or more commonly by the use of potassium monopersulfate.



1,3-dibromo-5,5-dimethylhydantoin



1-bromo-3-chloro-5,5-dimethylhydantoin

–*Impact of pH on efficacy.* Hypobromous acid ionizes to form a hypobromite ion (OBr^-) to a degree that is less dependent on the pH of the water. For example at pH 7.0, 98% of the bromine is hypobromous acid and at 8.0 83.3% is available as HOBr. See Table 3 listed below.

The biocidal efficacy of hypobromous acid and hypobromite ion are nearly identical. This is in stark contrast with chlorine where the efficacy of hypochlorite ion is approximately 100 fold less than the acid.

–*Impact of sunlight on stability.* Bromine is more subject to dissipation in sunlight and will produce a less stable residual than chlorine. Currently there are no commercial bromine stabilizers used in recreational water treatment.

Table 3 The effect of pH on hypobromous acid concentrations in recreational water (Nalepa, C. J. 1997)

pH	Percent HOBr
7.0	98
7.5	94.1
8.0	83.3
8.5	61.3

–*Reactions with ammonia and organic compounds.* Hypobromous acid reacts with amines to form bromamines in a manner similar to chlorine. However bromamines are efficacious as sanitizers in recreational water, unlike chloramines which are biocidal, but of limited value due to their slow rate of kill. Additionally, bromamines are not irritating to the skin and eyes and do not possess a strong odor. “Breakpoint” bromination is not required. Re-oxidation of the inactive bromide ions will re-convert the salt to biocidal form (HOBr/OBr^-). These qualities make bromine biocides much more favorable products for use in spas, relative to chlorine. Bromine products are primarily used for treating indoor pools and spas. Chemicals used for the neutralization of hypobromous acid are identical to those used for hypochlorous acid.

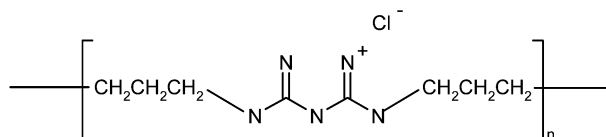
–*Test methods.* The same direct and indirect test methods used for the measurement of chlorine residual can be used for measuring total bromine. The results of the chlorine assays must be multiplied by a factor of 2.25 to account for the difference in the chlorine and bromine molecular weights. In bromine systems the only active reported is the total bromine. This is a reflection that combine bromine (bromamine) is efficacious.

5.3.2.2 Polyhexamethylene biguanide [18.3.3]

Biguanides are a class of man-made compounds that have been used as antimicrobial agents in the preservation of cosmetics and pharmaceuticals for over 50 years. The historical development of PHMB has been summarized by May (1991). Biguanides were first used as malaria treatments. It was later discovered that polymeric biguanides had greater bactericidal activity, particularly polyhexamethylene biguanide (PHMB). ICI Americas (now Avecia, Ltd.) patented the use of PHMB in swimming pools in 1977 (Carter and Hinton, 1977). The US Environmental Protection Agency registered PHMB for use as a swimming pool sanitizer and algistat in 1982. The first commercial use in US pools was in 1983. In 1993 the US EPA registered PHMB as a sanitizer for spas. PHMB is the only non-halogen, non-metallic biocide registered by the US EPA as a sanitizer for recreational water.

The mode of action of PHMB is through permeabilization of the cytoplasmic membrane. The cationic biguanide units are electrostatically attracted to anionic head groups in the outer leaflet of the cytoplasmic membrane. The hexamethylene linkage groups partially insert into the outer leaflet. This results in repulsion of adjacent anionic phospholipids with consequential disruption in the packing of the phospholipids hydrophobic tail units. The disruption of the hydrophobic tail groups increases the permeability the membrane low molecular weight cytoplasmic components, including K^+ ions (May, 1991).

PHMB is a highly water soluble polyelectrolyte. The structure of PHMB is shown below.



where average $n=12$

PHMB – poly(hexamethylene biguanide) hydrochloride.

PHMB is initially dosed at 10 ppm active (50 ppm product) and maintained in pools and spas between 6–10 ppm active (30–50 ppm as product). It is typically consumed at a rate of 0.4 ± 0.2 ppm/day. This can vary as a result of environmental factors. It is topped-up when the level is at or below 6 ppm. This is accomplished about every 7–14 days.

The chemistry of PHMB stability is in sharp contrast with that of halogen based systems. Unhoch et al. (1996) compared the stability of PHMB to that hypochlorous acid under the environmental conditions that occur in recreational water. PHMB was shown to be stable at temperatures 39°F and 108°F, unaffected by pH ranges from 6.2 to 10.0, and lost less than 10% active after continuous exposure to artificial sunlight. Unstabilized hypochlorous acid was shown to have significantly reduced stability or activity under the same conditions. PHMB is not significantly affected by the daily accumulation of organic materials and does not react with common pool and spa organic compounds. PHMB reacts preferentially with proteins and fatty acids found in the bacterial membrane and only slightly with other organic compounds found in pool and spa water. This difference in reactivity was shown to have practical implications in a study of simulated spa usage. PHMB was shown to have significantly improved control of heterotrophic counts, coliforms and *Pseudomonas aeruginosa* over that of chlorine in the presence of moderate organic loading (Vore, 1997).

One property of PHMB that has received some consideration is the rate of kill. The AOAC method 965.13 measures the rate of kill of recreational water sanitizers under laboratory conditions in system devoid of organic

matter. Hypochlorous and hypobromous acid displays complete kill in the method at 30 seconds. PHMB, at use concentrations, displays a compared complete kill in three to five minutes (Vore, unpublished data). The significance of this method has never been linked to performance under conditions of use. When the efficacy of sanitizers is assessed in the presence of organic matter PHMB, hypochlorous acid, and hypobromous acid display remarkably similar complete kills (Vore et al., 1997).

PHMB because it is not an oxidizer will not bleach pool vinyl surfaces, degrade fabric or cause colors to fade in swimwear (Unhoch and Vore, 1996). An oxidizer is needed to assist in controlling the accumulation of organics resulting from bathers and the environment. The preferred oxidizers for use with PHMB are peroxides, peracids, and perborates. Ozone can also be used to control the accumulation of organics in pool and spa water. Chlorine products cannot be used because they react with the PHMB removing it from the water as a sticky yellow precipitate.

Other commonly used pool and spa chemicals incompatible with PHMB are anionic surfactants, phosphate-based chelating agents and copper algicides.

PHMB concentrations in pools and spas are measured by the consumers either using a liquid reagent visual colorimetric test where the color of the sample is matched with a chart, colorimetric test where it is measured using a colorimeter (dual wavelength spectrophotometer) and test strips. Pool and spa owners commonly use test strips to monitor PHMB whereas; pool and spa dealers use the colorimeter. The technology is identical to that used for chlorine except a different reagent system is used. ORP is not an appropriate method for PHMB because PHMB is non-oxidizing. Further, PHMB systems use hydrogen peroxide as the oxidizer. At the concentration of hydrogen peroxide used (< 50 ppm AI) hydrogen peroxide does not appreciably modify the redox potential of the water.

5.3.2.3 Silver

Silver has been employed as a recreational water biocide in limited quantities. Colloidal silver (Ag(II), particle size of 0.001 to 0.01 μ m) is presently registered by the US EPA as a primary sanitizer. The sole registrant and manufacturer is not presently promoting the product (Jonas Company, personal communication). This reportedly is due to silver staining of pool and spa surfaces. Several manufacturers market cartridge devices that release silver ions either through erosion or electrolysis (see section 2.5.5.).

5.3.2.4 Iodine [II, 21.2.12]

Iodine was used in limited quantities as a primary sanitizer from the 1950's into the 1980's. The manufacturers did not renew the US EPA registration during product review and the registration was dropped. Iodine is no longer employed as a biocide in recreational water.

5.3.2.5 Chlorine generators and supplemental sanitizers

The recreational water industry is presently observing an expansion of the number of automated biocide delivery systems as well as an increase in the number of biocides that provide supplemental sanitizer capacity. This expansion is not globally uniform. Similarly, the regulatory status of these biocides and treatments is not uniform. Below is a short summary of several systems that are widely available.

5.3.2.5.1 Chlorine generators (chlorinators). Electrolytic chlorine generators are available in a number of device configurations. These onsite systems generate the sanitizer by passing a low voltage direct current between electrodes and converting a brine solution into chlorine gas and sodium hydroxide. The systems vary considerably in design, but the eventual product of all designs is hypochlorous acid. The more advanced designs include automation using ORP and pH probes. One major disadvantage of these systems is that they require constant pH adjustment or safe disposal of the sodium hydroxide by-product (NSPI, 2000).

5.3.2.5.2 Ozone. Ozone is a strong oxidizer formed by the excitation of molecular oxygen into atomic oxygen in an environment that allows it to recombine into O₃. Ozone is used as an oxidant and supplemental sanitizer in both pools and especially in spas. The half-life of ozone is on the order of a few seconds (Haas, 1991). Because of the instability, it is not possible to maintain an ozone residual in the entire water column. Ozone must be used in conjunction with a primary sanitizer. Hypochlorous acid, hypobromous acid and PHMB are suitable as primary sanitizers for ozone system, although the consumption of PHMB will be increased by 25 to 50 percent (Unhoch and Vore, unpublished data).

Ozone may either be generated from atmospheric air or pure oxygen excited by means of electrical discharge (corona discharge, commonly referred to as CD) or photochemical action using ultraviolet light (commonly referred to as UV ozone). Corona discharge using atmospheric air is the method most commonly encountered.

Ozone is an unstable pungent smelling gas. It must be generated on-site. It is water soluble at 570 mg/L at 20°C. This is well above the use levels required for treating pools and spas.

The free radicals that form upon decomposition also possess great oxidizing power and are very reactive with organics present in the pool or spa environment. Ozone will oxidize bromide ion to bromine and bromate, chloride ion to chlorine and hydrogen peroxide forming hydroxyl radicals.

Determination of ozone residuals in water is quite difficult because of its rapid decomposition, volatility and reactivity. Most test methods available are variations of existing chlorine residual methods that are not specific for ozone.

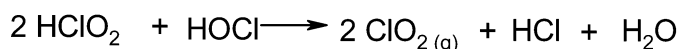
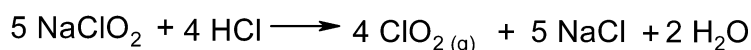
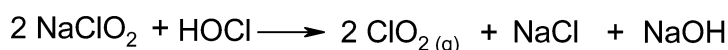
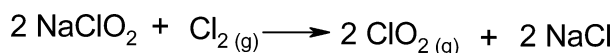
5.3.2.5.3 Ultraviolet light (UV). Ultraviolet Light (UV) is increasingly being used as a sanitizing treatment for recreational water. Because UV does not impart a residual into the water it must be used in conjunction with a primary sanitizer (hypochlorous acid, hypobromous acid, or PHMB). Because UV will increase the consumption rate of the primary sanitizer, the concentration of the primary sanitizer must be monitored more closely and the feed rate of primary sanitizer adjusted accordingly.

UV results in degradation of genetic material. The most efficacious wavelengths are about 260 nm. The most commonly used lamp design is low-pressure mercury vapor lamps, which have a monochromatic output of 254 nm. At this wavelength the light forms dimers between adjacent thymine nucleotides in DNA chains. The resulting thymine dimers inhibition the correct replication and transcription of the DNA (Friefelder, 1987). The role of pH in UV action has not been demonstrated (Haas, 1999).

UV units work by passing the water through a reaction chamber containing a UV lamp. The systems used in recreational water are quite simple compared to multiple lamp units used for potable water. The efficacy, durability, and longevity of present recreational water UV units have not been documented in a peer-reviewed format.

A number of UV/hydrogen peroxide systems are presently available. These systems specify a concentration of hydrogen peroxide system in the water column that does not provide a rapid kill of bacteria. Systems of this design lack a primary sanitizer in the water column. Suitable efficacy studies of UV/peroxide systems are not available and the US EPA has not registered such a system for use. Until suitable studies are published and the regulatory approval is granted, the use of UV/peroxide systems is strongly discouraged.

5.3.2.5.4 Chlorine dioxide [II, 21.2.4]. Chlorine dioxide was introduced as a recreational water supplemental biocide and oxidizer in 2002. Chlorine dioxide is an unstable, pure yellow-green gas with a pungent, irritating odor similar to other chlorine products. It is soluble in water, but does not undergo hydrolysis. Reacting sodium chlorite with gaseous chlorine or sodium hypochlorite and hydrochloric acid normally generates chlorine dioxide on-site. There are other ways to generate chlorine dioxide. The reactions are listed below (Gates, 1998).



Generation of chlorine dioxide

The attraction of this compound for recreational water treatment is related to its properties versus hypochlorous acid: strong oxidizer, efficiency is pH independent, does not react with ammonia nitrogen or primary amines, ability to penetrate and disperse biofilm, and reduction in the formation of reaction by-products like haloorganics and trihalomethanes. The disadvantages of chlorine dioxide use in pools and spas are that it imparts a yellow-green color to the water at use levels, can be degraded by sunlight and is volatile in re-circulating water systems. The volatility and instability to sunlight will make it extremely difficult to maintain residuals essential for swimming pools. Because a residual of chloride dioxide cannot be maintained in the water, it cannot be used as a primary sanitizer. The system introduced in 2002 uses chlorine dioxide as a treatment for the biofilm accumulations in the circulatory system of PHMB sanitized pools and spas (Brown, et al., 1998).

The DPD method described above for measuring free chlorine can be used to measure chlorine dioxide in the absence of other oxidizers. If chlorine is present the DPD method can be modified to include treatment of the sample with glycine. This will convert the free chlorine to combined chlorine (chloroaminoacetic acid). It is important to note that any reading obtained for method is for chlorine and must be converted to mg/L or parts per million of chlorine dioxide by multiplying the result by 1.9. Chlorine dioxide can also be measured indirectly using ORP also described above.

Chlorine dioxide is a mucosal irritant: acute exposure can cause eye, nasal, throat, and lung irritation. Prolonged exposure can cause bronchitis, reactive airways disease and pulmonary edema. Its health effects are not cumulative. Chlorite is one of its disinfection byproducts can be capable of inducing hemolytic anemia and methemoglobinemia also commonly associated with oxidative stress.

5.3.2.5.5 Copper/silver ionizers. An ion generator passes low voltage direct current between two electrodes releasing copper (Cu^{+2}) and silver (Ag^{+}) ions into the pool water. The current controls the quantity of ions released to the water. The silver ions are bactericidal. The copper ions are present to control and/or inhibit algal growth. The ionizers do not oxidize organic matter in the pool and therefore, must be used with oxidizers. Because of the slow kill rate of silver, these systems must be used with a halogen primary sanitizer. NSF International Standard 50 requires copper/silver ionizers to have a minimum of 0.4 ppm free available chlorine residual in the water (NSF, 1996). The water should be tested periodically to monitor silver and copper ions, as well as, free available chlorine residual. In reality, tests for silver and copper are seldom done in the residential market. Copper and silver ions are both subject to oxidation leading to precipitation of the corresponding salts causing staining of pool surfaces.

The regulatory status of ionizers, and similarly constructed passive metal release systems, is questionable. These systems specify in their use directions concentrations of chlorine that are in conflict with the directions approved by the EPA.

5.3.3 Algicides and fungicides

Algal infestations or blooms primarily occur in swimming pools and to a much lesser degree in stand-alone spas. The use of algicides in stand-alone spas is rare. Recreational water algae, unlike bacteria, are not pathogenic. Algae are unsightly and present a hazard through slippage and obscuring vision. Vision is vital in quickly locating floundering swimmers. The recreational water industry classifies algae by color. Green algae (*Chlorella spp.*) are the most common. Green algae are fast growing planktonic algae that usually first appears as cloudy or hazy water that within hours turns to green. Green algae are also the easiest to control. The second most common algae are mustard or yellow colored (*Eustigmatos spp.*). These sessile algae are much more difficult to control because they attach to rough surfaces like the plaster pool walls or in cracks or the folds of a vinyl liner. The least common algae are black in color (*Phormidium spp.*). Black algae are sessile and difficult to control for the same reasons as the mustard algae. Black algae have not been reported in PHMB-sanitized pools (Vore, unpublished data).

Algicides are routinely added, usually weekly, as preventative treatments. Most algicides also have label directions for remedial treatments, usually at about two-fold greater concentration than those called for in preventative treatments. Comments on use concentrations in the section below were taken directly the EPA approved use directions.

Fungal growth may lead to system infestations. Vore (1998) reported a complex consortium of bacteria and fungi in residential pool filter beds. The organisms reported are of limited concern to operators and users. Fungal outbreaks in PHMB-treated pools have been noted. The putative organism is *Paecilomyces lilacinus* (Vore, unpublished data). Mycelial mats of this organism begin as white in color and shift to pink as the colony matures. In full-blown out breaks the mycelial sheets may cling to the poolsides and resemble sheets of tissue paper. To treat this problem AVecia Inc. introduced a patented synergized PHMB blend (trade name Baquacil Ultra) in 2001 and Biolab Inc. introduced a chlorine dioxide treatment (trade name Assist) in 2002. Reviews of the efficacy of these systems have not been published to date. For proprietary reasons the only available data on these treatments is in their respective patents (Unhoch, 1995 and Brown, 1998).

5.3.3.1 Alkyl dimethyl benzyl ammonium chloride [II, 18.1.2]

Alkyl dimethyl benzyl ammonium chloride (ADBAC) is the most commonly employed recreational water algicide. Most sanitizer system directions suggest a slug addition of an algicide at pool opening coupled with a weekly top up addition of the algicide. The target concentration is generally between 2 and 4 ppm (active) range. Above 4 ppm foaming becomes a concern, especially in pools with fountains, waterfall features or pools sanitized by PHMB. ADBAC quaternary ammonium compounds (quats) are effective against green algae, but not against mustard algae. The MIC of ADBAC's against field-collected mustard algae is frequently above 50 ppm (Vore, unpublished results). ADBAC quats are sold as aqueous liquids ranging in strength from 10 to 50% active.

5.3.3.2 Poly(oxy)ethylene(dimethylimino)ethylene(dimethylamino)ethylene dichloride [II, 18.1.11]

Poly(oxy)ethylene(dimethylimino)ethylene(dimethylamino) ethylene dichloride (WSCP), or poly quat, is the "second most commonly quat in recreational water. The primary advantage of poly quat is that it produces

no foam at use concentrations, even when used in pools with fountains, water falls or in combination with PHMB sanitizers. Poly quat is used at 8 to 13 ppm active. The MIC of poly quat against resistant mustard algae is generally in the same range as ADBAC quats (Vore, unpublished data). Formulations employed vary in concentration from 10 to 60% active. Buckman Laboratories and Avecia Inc. combined to launch a re-formulated poly quat during 2001. This formulation is noteworthy for its efficacy against mustard algae in PHMB treated pools. This formulation is not compatible with halogens and cannot be employed in chlorine treated pools.

5.3.3.3 *Dodecyl dimethyl ammonium chloride*

Dodecyl dimethyl ammonium chloride (DDAC) has seen limited use in recreational water. The primary advantage of DDAC is that it is efficacious at the same use concentration as ADBAC but has a greatly reduced tendency to foam. DDAC is used at 2 to 4 ppm active.

5.3.3.4 *Chlorine*

Chlorine release biocides are frequently employed as remedial algicides. Many, but not all, of the chlorine products used as primary sanitizers also have directions for use as algicides on their labels. These include chlorine gas, liquid bleach, calcium hypochlorite, and dichlor. To effectively function as an algicide it is necessary to raise the free chlorine concentration to above 5 ppm (superchlorination). The higher concentration is required to meet the chlorine demand associated with the large quantity of organic material produced during algal blooms. Trichlor is not an effective algicide because its rate of dissolution is too slow to serve as a superchlorinator.

5.3.3.5 *Copper compounds*

Copper algicides are frequently employed in halogenated pools. Compounds used include copper sulfate and copper triethanolamine. Copper algicides are noteworthy for their efficacy against mustard algae species in halogenated systems. Copper algicides are not suitable for use in PHMB systems. Copper ions reacting with PHMB result in the formation of elastic lavender colored precipitate (Unhoch, unpublished data). Copper ions are subject to oxidation leading to precipitation of the corresponding salts causing staining of pool surfaces.

5.3.3.6 *Sodium bromide*

Sodium bromide is occasionally employed as an algicide. Typically 3.5 ppm of sodium bromide added. The bromide is converted to hypobromous acid by the subsequent addition of a fast dissolving chlorine biocide sufficient to achieve 10 ppm FAC. This temporarily converts the pool to a bromine system. The pool is reconverted to chlorine by subsequent high doses of chlorine release agents.

5.3.3.7 *Silver*

Silver and silver salts have occasionally been employed as algicides. Silver based algicides have shown limited efficacy against mustard algae and are frequently cited as the source of stains of pool surfaces.

5.3.4 **Oxidizing agents**

The role of oxidation in the proper operation of pools and spas has gained much greater understanding during the past decade. The NSPI's Chemical Operational Parameters now includes a separate section just on oxidation (NSPI, 2002A). Commonly employed oxidants include chlorine biocides, potassium monopersulfate (MPS), "hydrogen peroxide [II, 21.1.1.], sodium perborate [II, 21.1.2.], and sodium percarbonate. These compounds, with the exception of chlorine products, are used at concentrations too low to be biocidal. MPS is used in halogen-treated waters. Up to 90% of the chlorine used in recreational water is consumed during oxidation of organic contaminants (White, 1972). If used weekly MPS is intended to oxidize the organic material and reduce the amount of chlorine required to maintain an acceptable sanitizer in the water column (Lightcap, 1996). MPS is normally used at 12 ppm per week. Because PHMB is a non-oxidizing biocide a separate oxidizer must be employed. The preferred oxidation system for PHMB treated pools is hydrogen peroxide, which is normally added at 27 ppm active on a monthly basis. Sodium perborate and sodium percarbonate have been used as supplemental sources of hydrogen peroxide in PHMB systems. These are used at 8 ppm to over 50 ppm. Neither peracid should be considered a stand-alone oxidizer due their minimal generation of hydrogen peroxide. Additionally, both of these compounds elevate the pH of the water.

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5.4 Oilfield application for biocides

D.B. McILWAINE

5.4.1 Introduction

Oil and gas production occurs throughout the world. Each oilfield is unique and the construction and development of each new oilfield presents many challenges and opportunities for petroleum engineers. Differences in geology, topography, and climate in and around an oilfield will dictate how the oilfield is developed, maintained, and eventually decommissioned. However, one thing that is a constant in an oilfield, regardless of its location, is the need for biocides. Biocides are used in all stages of oilfield development, from the initial drilling of the wells and the day to day production of oil and gas, and in all aspects of the maintenance of the field. They play an important role in the life of an oilfield and are a valuable tool in ensuring that oil and gas are produced safely and reliably. This chapter will describe various aspects of the use of biocides in the production of oil and gas.

The world's oilfields

Petroleum production is currently on going on six of the seven continents of the world. Given the increasing demand for energy, it is probably only a matter of time until oil and gas exploration starts in the wilderness of Antarctica. However, until then the world's oil and gas supply is being supplied from existing fields in the following areas. In the America's, the fields of Alaska's North Slope supply crude via the TransAlaskan pipeline to refineries in California and Asia. New deepwater fields in the Gulf of Mexico are posing significant technological challenges for oil companies from both a production and environmental point of view. Oil and gas fields in Western Canada's Alberta province, supply fuels to both Canada and the US. Among the newest fields in the Western Hemisphere are the gas fields of Sable Island, off the coast of Nova Scotia, and the Hibernia and Terra Nova oilfields off the coast of Newfoundland. New fields are currently under construction, and exploration continues in the Georges Bank area off of Eastern Canada. Central and South America boasts oil and gas production from Mexico, Venezuela, Argentina, and Brazil.

Major European fields can be found in the North Sea area and the producing countries include the United Kingdom, Denmark, and Norway. The Middle East countries continue to supply a significant amount of the world's energy from fields in Saudi Arabia, Iran, Oman, Egypt, and Syria among others. Africa produces oil and gas from countries such as Algeria, Libya, and Nigeria. Finally, Asia Pacific countries that produce oil and gas include Australia, India, Indonesia, Malaysia, and China.

The interested reader can follow the worldwide oilfield drilling and production activities by monitoring the Baker Hughes rig count as found on their website: www.bakerhughes.com/investor/rig/index.htm. The active rig count acts as an indicator for the demand of those products used in drilling, completing, producing and processing of natural gas and petroleum products. Further information on the significance of the rig counts can be found on this website.

5.4.2 Description of an oilfield

A simplistic view of an oilfield would be that it consists of two main parts; the injection system and the production system (Bradley, 1987). Before discussing the importance of each of these systems, some definitions are required that will be helpful in understanding oil and gas production.

Stages of oil production

When discussing oil production, the following terms are commonly used.

1. *Primary production* – Primary production relies on the natural pressure in the oil and gas bearing formation to force the hydrocarbon product out of the ground and into a gathering system. The very first oil wells drilled in the United States in the late 1850's relied on the pressure in the formation to produce the oil from the ground. No currently operating oil fields rely on primary production.
2. *Secondary production* – Secondary production, or waterflooding, involves the injection of water or into the reservoir to maintain sufficient pressure to force the oil out of the ground. As the injected water flows through the formation, it helps to flush the oil from the formation and into the production wells. Most oilfields operating today use secondary production to produce their oil and gas.

3. *Tertiary production or enhanced oil recovery (EOR)* – EOR is usually the most expensive method of recovering oil and involves the use of polymers, gases such as carbon dioxide and nitrogen, or the use of steam to force the remaining oil out of a formation. Due to the high cost of EOR, there are few fields operating today using solely EOR as the method of production.

5.4.2.1 The injection system

The injection system is the portion of the field that is responsible for injecting water (in the case of a waterflood) into the formation. The source of the water that is injected may be a surface water source, such as a lake, river or ocean. If this water has never been injected downhole or come in contact with hydrocarbons, it is referred to as fresh water. Water that is produced from the formation along with oil and gas is referred to as produced water. Produced water is separated from the oil and gas by the action of a series of separators in the production system. The production system will be discussed in detail below. A simplistic diagram of an injection system is shown below in Figure 1.

The injection water is usually treated to address several concerns. If the oilfield is large enough and the injection water is taken from a surface water source, it may be treated with an oxidizing biocide as it is gathered in order to kill any planktonic (or free-floating) microorganisms that may be present. It is common for oilfields that use seawater for injection to generate chlorine on-site via electrolytic methods to treat the injection water. The injection water is then deaerated to remove dissolved oxygen. Dissolved oxygen in the water is a concern since it could lead to an increase in the rate of corrosion of the pipelines of the oilfield. Therefore, the water is deaerated either by a vacuum tower, by passing natural gas through the water, or by using a chemical oxygen scavenger such as ammonium or sodium bisulfite. It is also common for a combination of these methods to be used to remove dissolved oxygen to keep the field anaerobic. After deaeration, the injection water may then be treated with a nonoxidizing biocide prior to its injection downhole. The site of addition of the nonoxidizing biocide is especially important. Since the deaeration tower is often a breeding ground for bacteria, many fields inject the biocide into the basin of the deaeration tower (Maxwell, 2002). This biocide treatment ensures that no viable microorganisms are introduced into the formation.

There are several criteria that a biocide for injection water must meet.

1. *Stability.* The biocide must be stable in the injection water long enough that it is active once it is injected downhole. Since most surface waters are neutral to slightly alkaline in pH, care should be taken to use a biocide that is stable at pH's of 7 and above. Also, it is possible that the distance from the water treatment plant to the injection wells could be quite far. Hence, stability and activity of the biocide in the injection water for a period of several days may be required to ensure effectiveness. In Alaska for example, the distance from the Kuparak Seawater Treatment Plant to the Alpine field is approximately 25 miles. A short-lived biocide would not be effective in oilfields where there is a large distance from the treatment plant to the injection wells.
2. *Compatibility with other system additives.* This is another important requirement for the injection water biocide. The most common concern about chemical incompatibilities is an interaction between the biocide and the oxygen scavenger. Most commercially available biocides for oilfield use will react with the bisulfite based oxygen scavengers and become deactivated. This interaction will be discussed in greater detail in the discussion of the commonly used oilfield biocides.
3. *Efficacy.* With some biocides, notably the quaternary ammonium biocides, there are concerns about the hardness of the water affecting the efficacy of the biocide. This concern generally stems from the observation

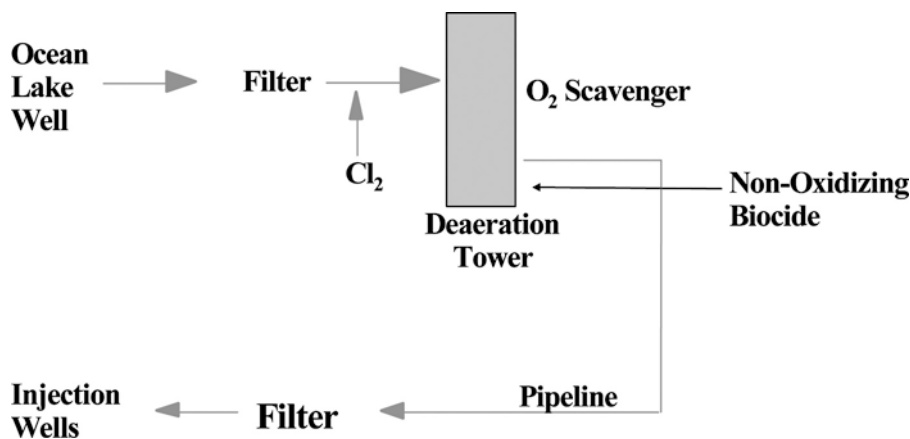


Figure 1 Diagram of the injection system of a water flood oilfield.

that some quats may precipitate from solution if added into a high salinity brine. Therefore, it is imperative that the effectiveness of the biocide be evaluated prior to its introduction in the field.

4. *Other considerations.* Other considerations for an injection water biocide include whether the system can tolerate a biocide that foams. Quaternary ammonium compounds are effective biocides but some quats have a tendency to foam. Lastly, safety and handling considerations are very important in the choice of a biocide. All biocides should be handled with extreme care so it is critical that the choice of a biocide includes a complete and thorough evaluation and understanding of the safety requirements detailed in the Material Safety Data Sheets for each product.

5.4.2.2 The production system

The production system is that part of an oilfield that is responsible for separating the produced fluids, consisting of natural gas, oil and water, into their component parts (Gray, 1986). The separation process will usually consist of heating, gravity, mechanical methods and any combination of these. A simplistic schematic of a production system is shown below in Figure 2.

The produced fluids from all of the producing wells in the field are gathered into a larger stream prior to separation. These fluids contain gas, oil, water and other impurities such as iron sulfide, sulfate scales (barium sulfate, calcium sulfate), sand and other insoluble particulates. While the natural gas is generally easy to separate for the other components, the oil and water that are produced usually exist as an emulsion. It is through the action of the separators (usually with the aid of chemicals) that the emulsion is broken into oil and water. A description of the separation process is as follows.

1. The primary separator may also be known as a heater/treater tank. Heater/treater tanks are usually vertical tanks but some are horizontal. In a vertical heater/treater, the produced fluids flow into the top of the heated tank where they fall to the bottom and are circulated through various baffles or other features in tank. The gas generally separates from the mixture and is taken off the top of the tank. The water in oil emulsion is then mechanically mixed and some separation occurs. An oil take-off valve is usually located in the upper half of the tank that allows any free oil to be drained off. A drain in the bottom of the tank also will allow any free water to be taken off as well.
2. The emulsion that remains in the heater/treater is then sent to a secondary separator which may be referred to as a free water knock out. This type of separator may or may not be heated and is usually horizontal.
3. The last piece of the separation train may be called a gunbarrel tank and is a vessel that allows gravity and time to allow the oil/water emulsion to separate. The oil is taken off the upper portion of the tank and the water is withdrawn from the bottom. This water, called produced water, is either disposed off, or reinjected into the formation.

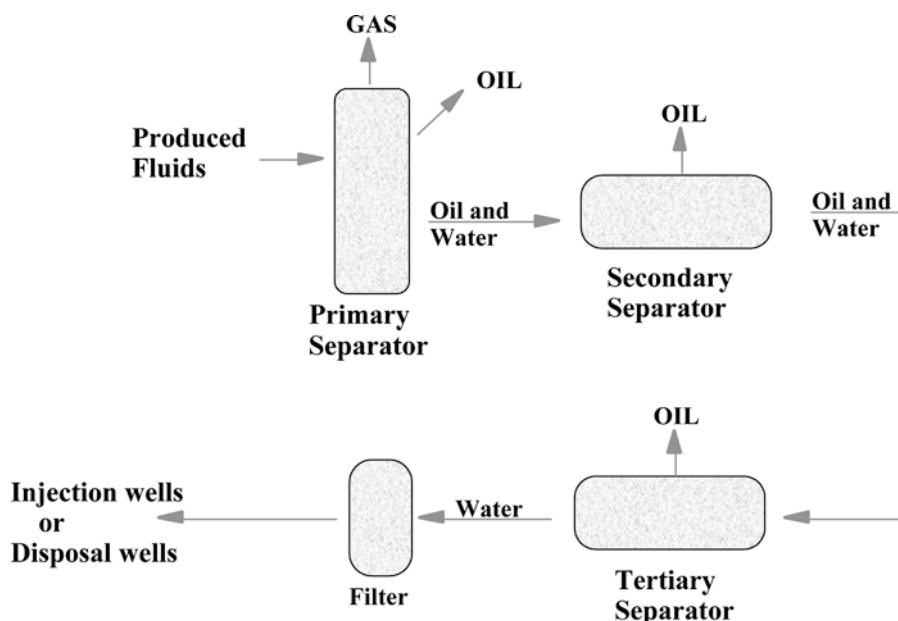


Figure 2 Diagram of the production system of an oilfield.

Problems in the separation system. There are several problems that oilfield operators and service companies address in production system of an oilfield. These problems may involve solids that inhibit or reduce the efficiency of the separation process, or they may be microbiological in nature. The solids that present the most problems in the separation system are either sulfate-based scale, namely calcium or barium sulfate, or iron sulfide.

Scale. The sulfate based scale solids are usually formed when injection water that is high in sulfate ions is injected into a formation that contains high concentrations of carbonates (for example calcium carbonate). Scale formation also occurs in oilfields where fresh water (usually seawater) is mixed with produced water prior to injection. Scale formation can reduce oil production rates by plugging the pores of the formation, and it can reduce the efficiency of the separation system. In the case where scale has formed near the bottom of the producing well, several remedial treatments are commonly used to dissolve the scale. These treatments are called squeeze treatments and involve the pumping of treatment chemicals down a production well and into the oil-bearing formation. The most common squeeze treatment involves the use of acid to dissolve the scale, or the use of proprietary scale inhibitors to prevent the precipitation of the scale. In the above ground equipment, the oilfield service companies generally try to control scale formation in the production system by the use of proprietary scale inhibitors such as organic phosphonates and low molecular weight polyacrylates. It is also the case that the use of certain chemicals in the production system can lead to the formation of sulfate scales. The use of the biocide THPS (Tetrakis(hydroxymethyl) phosphonium sulfate) in a squeeze operation was shown to lead to calcium sulfate precipitation when it was used in a formation that was predominately calcium carbonate (Nasr-El-Din, 2000).

Iron sulfide. Iron sulfide is formed by the reaction of hydrogen sulfide with iron containing compounds naturally present in the formation, the injection water, or from the metal components of the oilfield equipment. The source of the hydrogen sulfide can either be natural or by the action of microorganisms called sulfate-reducing bacteria. How these bacteria accomplish this will be explained more fully in the section on Oilfield Microbiology. Iron sulfide fouling can also decrease oil production rates by plugging the formation. However, it usually is more problematic in the separation system. Iron sulfide is known as an oil-wet solid and can contribute to the formation of emulsions at the oil and water interface. These emulsions can be very difficult to break and lead to incomplete separations of the oil and water. Few effective methods are available to address iron sulfide fouling although hydrochloric acid squeeze treatments have been used to dissolve iron sulfide down-hole. This procedure suffers from the fact that it is extremely corrosive to the metals in the system, generates large quantities of hydrogen sulfide gas, and requires the use of chelating agents to ensure that insoluble metals salts do not precipitate out of the acidic solution. In recent years an alternative treatment has been shown to be effective against iron sulfide in production systems. The use of the biocide THPS has been shown to be an effective in dissolving iron sulfide and improving separation efficiency (Walker, 1991; Diaz, 1998; Nasr-El-Din and Al-Humaidan, 2001). The nature of this reaction is complex but is an organometallic chemical reaction involving the active form of THPS, THP or trishydroxymethyl phosphine, with the iron sulfide. Since there are many forms of iron sulfide, the exact structure of the reaction product is not known, although a reaction product has been isolated and characterized (Jeffery et al, 2000; Gilbert et al, 2002).

Microbiological contamination. The separation system is often subject to microbiological contamination. The produced fluids often contain sand and dirt, in addition to the scale and iron sulfide noted above. The presence of these solids can lead to the accumulation of large quantities of insoluble material in the bottom of the various tanks in the separation system. These deposits then serve as a breeding ground for anaerobic microorganisms such as SRB's. The presence of SRB's can lead to H₂S formation and increased corrosion rates. It is often very difficult to address microbiological contamination in the production system because of several factors. First, the presence of the solid accumulations in the bottom of the separators makes it difficult for a biocide to penetrate and kill the microorganisms contained within. Second, a production system can be considered a once-through system, meaning that the water in the system is not recirculated (as is the case in a cooling tower system for example) and contamination is continuously being re-introduced. A slug treatment of biocide has a limited residence time in a separation tank before it passes on in the system. For these reasons, it is difficult and costly to treat a separation system with a biocide. Biocide treatments may be performed on a continuous basis with a low concentration, or slug doses on a regular schedule. Either way, these treatments are often accompanied by cleaning procedures in which the sand and other insoluble material is removed from the bottoms of the separators.

5.4.3 Oilfield microbiology

The oilfield is an interesting place for microbiologists. The conditions encountered range from cold to hot, and from fully aerobic to anaerobic. These conditions breed different types of microorganisms that have adapted to

the environments in which they live. While some microorganisms present no problems for oilfield operators and service companies, others are extremely problematic and their presence can lead to a multitude of problems. What follows is a brief description of the types of organisms that inhabit the oilfield and the concerns that exist over their presence.

Sulfate reducing bacteria

Sulfate reducing bacteria (SRB's) are common in the oilfield and have been the focus of much of the efforts of biocide manufacturers and service companies due to the problems that they cause (Postgate, 1984). SRB's are anaerobic bacteria, but they can be found in waters that contain dissolved oxygen (Tatnall, 1993). The most common of the SRB's are of the *Desulfovibrio* genus, but others are also known. They can be found in most oilfield waters and are tolerant of pH's from 5 – 9, and temperatures from 25° C to 60° C. Thermophilic strains of SRB's are also known to grow at temperatures above 60° C (Antolga and Griffin, 1985; Stettar, 1993).

These organisms are problematic in the oilfield since their metabolic pathways involve the reduction of sulfate to sulfide. Sulfate is present in all water to some extent but especially in seawater where it may be present at concentrations of greater than 2000 parts per million. The biogenic sulfide that is produced by the SRB's may react with soluble iron to form insoluble iron sulfide that precipitates out of solution and can cause problems in the separation system. Concurrently, the sulfide, if combined with protons to form hazardous hydrogen sulfide, may also be responsible for souring of oil and gas and for the corrosion of metal surfaces. This corrosion is called microbiologically influence corrosion (MIC) and the control of MIC is the most compelling reason for the use of biocides in the oilfield. The mechanism of MIC involves a series of electrochemical reactions that are the result of the metabolism and the metabolites of the SRB's. Much work has been done in this area and the references cited will provide the reader with valuable and detailed information with regards to the mechanism of MIC (NACE, 1990; Borenstein, 1994; Geesey et al, 1994).

Iron and manganese oxidizing bacteria

Iron oxidizing bacteria also play a role in MIC of metal surfaces (Iverson, 1987). These organisms belong to several genera including *Gallionella*, *Siderocapsa*, *Sphaerotilus*, *Crenothrix*, *Leptothrix* or *Clonothrix*. These organisms are capable of oxidizing ferric ion (Fe^{2+}) to ferrous ions (Fe^{3+}) and manganous (Mn^{2+}) ions to manganic (Mn^{3+}) ions to obtain the necessary energy for their growth and survival (Tatnall, 1993). In oilfield systems, the presence of ferric or manganic ions can lead to the formation of ferric and manganic chloride, both of which are corrosive to carbon and stainless steels. These metal oxidizing bacteria are also able to scavenge oxygen and can help contribute towards creating a hospitable environment for anaerobic bacteria.

Acid producing bacteria

Acid producing bacteria (APB's) are those microorganisms whose metabolism results in the production of organic or inorganic acids. Examples of these organisms include *Clostridium aceticum*, that produces acetic acid, and *Thiobacillus thiooxidans* that produces sulfuric acid. Both of these organisms can contribute towards MIC of metal surfaces (Little et al, 1991).

Biofilms (Characklis and Marshall, 1990)

In any industrial water system, microorganisms will be found freely floating in the bulk water phase and attached to a surface. Those organisms that are free floating are called planktonic organisms and those attached to surfaces are termed sessile organisms. The conditions in which the organisms exist will dictate whether or not the organisms are planktonic or sessile (Geesey, 1993). Planktonic organisms will become sessile organisms upon their attachment to a submerged surface. Once attached, the organisms replicate on the surface and begin to produce a protective biofilm. The biofilm is composed of microbial cells and extracellular polysaccharide polymers often crudely referred to as slime. As the immobilized cells continue to grow and produce additional amounts of extracellular polymer matrix, the biofilm grows and entraps other microorganisms, corrosion by-products, and debris typically found in industrial waters. This process creates a complex community of organic and inorganic substances (Little, 1991). As the biofilm grows thicker, the diffusion of dissolved gases through the biofilm becomes more difficult and as a result, some cells will die. Both aerobic and anaerobic microbes can live in a biofilm with the aerobes usually found in the outer layers of the biofilm (near the bulk fluid) while the anaerobes are found in areas of low oxygen concentration, usually at the base of the biofilm. This diverse collection of microbes found within a biofilm is usually called a consortium (Little, 1991). Biofilms are especially troublesome in industrial applications since they are known to provide a protective environment for the growth

and proliferation of the entrained microbes. Research has demonstrated that sessile microorganisms are more difficult to kill with antimicrobials than are planktonic microbes (Grobe and Stewart, 2000; Stewart et al, 2000; Xu et al, 2000).

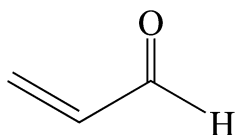
Problems caused by biofilms

The term biofouling refers to the formation of the biofilms on surfaces (Costerton et al, 1978; Characklis, 1981; Characklis and Marshall, 1990). Biofilms can cause several problems in industrial water systems [see also chapter 5.1.]. The colonization of heat exchange tubes or cooling tower fill can lead to decreased efficiencies in heat transfer systems whereas biofilm growth in pipes may contribute to wear and decreased performance of pumps due to the increased fluid frictional resistance. Human health can also be adversely affected by biofilm growth. Biofilms have been implicated as a contributing factor in *Legionella* outbreaks in cooling tower (McCoy, 1997). In these cases, it is thought that the biofilm provides a protective environment for the *Legionella* bacteria and the protozoa that may harbor the *Legionella* bacteria. Medical science is very concerned with the formation of biofilms on urinary catheters (Nickel et al, 1985a; Nickel et al, 1985b; Costerton and Stewart, 2000) while dentists' repair the damage to teeth caused by the buildup of dental biofilm called plaque (Costerton et al, 1999). In the oilfield, biofilms contribute to the corrosion of metal surfaces and to the souring of oil and gas. They do so by harboring the SRB's and APB that are responsible for sulfide generation and MIC.

5.4.4 Commonly used oilfield biocides

The following is a summary of the nonoxidizing biocides that are most commonly used in all aspects of oilfield operations. For each biocide, the structures, mechanism of action, and compatibility concerns will be discussed. It should be noted that all oilfield biocides are reactive chemicals that should always be handled with extreme care and in accordance to the manufacturer's instructions on the material safety data sheets (MSDS).

*5.4.4.1 Acrolein [II, 2.6.]**



Acrolein (2-Propenal)

CAS # 107-02-8

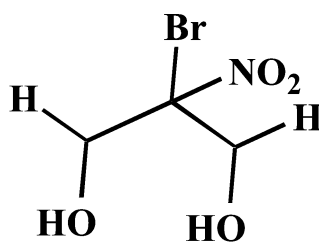
Acrolein is an extremely reactive molecule that is used to perform several different functions in the oilfield. It is an effective biocide at low use concentrations and will also reduce hydrogen sulfide concentrations. It is also used in squeeze treatments to aid in the dissolution of iron sulfide.

Mechanism of action. Acrolein has two functional groups that can contribute to its biocidal activity. It is an α , β -unsaturated aldehyde and as such the carbon-carbon double bond is extremely reactive. Nucleophiles, typically sulfur based nucleophiles, can react with the terminal carbon in a Michael type reaction (March, 1992), while the aldehyde group can undergo reactions typical of all aldehydes. From a biocidal point of view, those sulfur-based nucleophiles would include cysteine residues of the cell wall and those proteins associated with the cell wall. The amine containing amino acids (lysine and arginine) may also react with the aldehyde group of acrolein.

Compatibility concerns. Acrolein will react with and be deactivated by the bisulfite based oxygen scavengers. It will also react with primary and secondary amines and ammonia. Its reaction with hydrogen sulfide and iron sulfide is beneficial but also serves to reduce its biocidal effectiveness. Therefore, in oilfields where there is both microbial contamination and hydrogen sulfide souring, the demand on acrolein will be high. Acrolein generally shows good compatibility with the other commonly used oilfield biocides and could be used in combination with any of these other biocides.

* see Part Two – Microbicide Data

5.4.4.2 Bronopol (II, 17.14.)



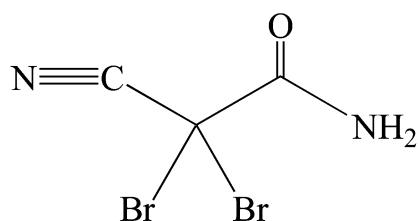
2-Bromo-2-nitropropane-1,3-diol
Bronopol
 CAS # 52-51-7

General information. Bronopol is a halogenated diol that also contains an electrophilic carbon atom. It is used extensively in water treatment applications and, like DBNPA (see 5.4.3.), has seen limited applications in the oilfield. It is used primarily in fluid preservation applications, most notable in the preservation of fracturing fluids or other fluids that are neutral to slightly basic in pH. Bronopol is particularly effective against *Pseudomonas* bacteria.

Mechanism of action. Bronopol is interesting in that it seems to function as a biocide via different chemical mechanisms depending on whether the system is aerobic or anaerobic. Under aerobic conditions, bronopol has been seen to catalyze the oxidation of cysteine amino acids to cystine residues (Shepherd et al, 1988). This action is thought to involve the formation of active oxygen species such as superoxide and/or peroxide which are likely responsible for killing the microorganisms. Further, the formation of the cystine residue crosslinks the cell wall, further hampering the normal functioning of the cell. The combination of active oxygen species and cell wall crosslinking results in cell death. Under anaerobic conditions, the likely mechanism of action involves the simple nucleophilic attack at the C-2 carbon by cellular and extracellular nucleophiles. Bronopol is generally not considered a fast acting biocide. Lastly, bronopol may release formaldehyde upon decomposition, but the formaldehyde is not responsible for its biocidal action.

Compatibility concerns. Bronopol will hydrolyze in aqueous solutions at varying rates depending on pH. The rate of hydrolysis is much faster at alkaline pH's than it is at acidic pH's. Increasing the temperature will increase the rate of hydrolysis at a given pH. The optimum pH range for Bronopol use is from pH 5–7. Bronopol will react with and become deactivated by sulfides and bisulfite based oxygen scavengers. Lastly, bronopol possess the potential to release nitrite that, in the presence of secondary amines, can form nitrosoamines.

5.4.4.3 2,2-Dibromo-3-nitrilopropionamide (DBNPA)[II, 17.5.]



2,2-Dibromo-3-nitrilopropionamide
 CAS # 10222-01-2

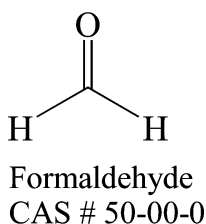
General information. DBNPA is a halogenated amide and is considered a fast kill biocide. It has seen widespread use in water treatment and pulp and paper applications but in the oilfield is used primarily to treat the water used to prepare fracturing fluids. Its main attribute seems to be that its biocidal action is very fast, but the active then hydrolyzes to less toxic by-products. This may be advantageous in applications where water is stored prior to discharge so deactivation may not be necessary.

Mechanism of action. The presence of two bromine atoms at C-2 of DBNPA means that this carbon atom is extremely electrophilic and will be the site of nucleophilic attack by cellular or extracellular nucleophiles.

The most likely cellular nucleophiles to attack the carbon backbone of DBNPA are the nucleophilic sulfur containing amino acids methionine and cysteine. The reaction is irreversible and the result is inactivation of the protein that contains these amino acids. It is also possible that the amine containing amino acids (lysine, arginine) may also react with DBNPA, but the rate of that reaction is probably slower than the reaction with the sulfur nucleophiles. This is due to the fact that at the pH range that DBNPA is most commonly used (less than pH 7.5) the amine groups of the lysine and arginine residues are protonated and will react slower than free amines.

Compatibility concerns. DBNPA will hydrolyze in aqueous solutions at varying rates depending on pH. The rate of hydrolysis is much faster at alkaline pH's than it is at acidic pH's. Increasing the temperature will increase the rate of hydrolysis at a given pH. The optimum pH range for DBNPA use is from pH 4 to 8. While it is possible to use DBNPA at pH's above 8, it is difficult to maintain any effective residual concentration due to its rapid decomposition. DBNPA will also react with, and be deactivated by, hydrogen sulfide and bisulfite based oxygen scavengers. DBNPA solutions are also not stable to UV light.

5.4.4.4 Formaldehyde [II, 2.1]

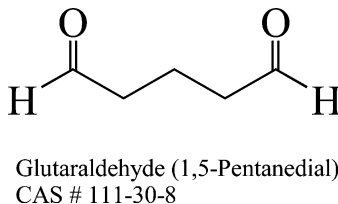


General information. Aqueous solutions of formaldehyde are generally used both as biocides and as hydrogen sulfide scavengers in the oilfield. It is very common for formaldehyde to be used in combination with quaternary ammonium compounds, glutaraldehyde and THPS (see 5.4.7.). Solutions of formaldehyde tend to be inexpensive (relative to the other commonly used biocides), so it continues to be widely used.

Mechanism of action. Formaldehyde mode of action has been extensively studied and is due to its ability to react with several different amino acids. It will react with those amino acids containing sulfhydryl (cysteine), hydroxyl (serine), and amine (lysine, arginine) groups. It is also unique in that it may also react with the purine and pyrimidine groups of both DNA and RNA. The varied nature of formaldehyde's mode of reactivity with all of these functional groups distinguishes it from other aldehydes such as acrolein and glutaraldehyde.

Compatibility concerns. Formaldehyde will react with and become deactivated by the bisulfite based oxygen scavengers (March, 1992). It will also react with hydrogen sulfide and so systems with microbial contamination and souring will place a high demand on the formaldehyde. In systems where formaldehyde is used in souring control, precipitation problems can occur (Walker, 1975).

5.4.4.5 Glutaraldehyde [II, 2.5.]



General information. Glutaraldehyde is a 5-carbon dialdehyde that has seen extensive use in industrial water treatment applications. In addition to its use as a biocide in oil and gas operations, it is also used in cooling water, paper making, and preservative applications, medical instrument sterilization, and as a non-biocidal crosslinker for leather, X-ray films, and enzyme immobilization. It is often used in oilfield applications in combination with other non-oxidizing biocides such as QAC's and formaldehyde and is compatible with the oxidizing biocides typically used in cooling water applications.

Mechanism of action. Chemically, glutaraldehyde is 1,5-pentanedial. As an aldehyde, it is a reactive molecule that undergoes those chemical reactions that are typical of any aldehyde. Its applications as a microbicide rely on the fact that it reacts with the free amine (non-protonated) form of primary and secondary amines in an irreversible manner. The pH of the surrounding medium, along with the pKa of the amine, will determine whether an amine is in its protonated or non-protonated (free) form. In acidic solutions, amines are generally protonated and their reaction with glutaraldehyde are slower than in alkaline systems where the amine is not protonated. For this reason, glutaraldehyde works faster in alkaline systems than acidic systems.

The mechanism of biocidal action of glutaraldehyde is undefined but probably involves its reaction with both the microbial cell wall and essential proteins associated with the cell walls. There are some twenty amino acids that make up all proteins; however they are arranged in many different sequences to form various kinds of proteins. Glutaraldehyde reacts primarily with only two of the twenty essential amino acids, lysine and arginine, since these two amino acids each have a free amino group. The reaction of glutaraldehyde with amino acids of proteins is recognized in the scientific literature to be an irreversible reaction although the exact composition and nature of the reaction product of glutaraldehyde and proteins still being debated (Kawahara et al, 1997). In order to speculate on the mechanism of the reaction of glutaraldehyde with proteins (and to form a hypothesis on its mechanism of biocidal action), it is helpful to first examine the reaction of glutaraldehyde with simple amines. Examples from the synthetic organic chemistry literature in which glutaraldehyde and a substituted amine are used as synthetic building blocks are useful in suggesting a mechanism for the reaction of glutaraldehyde with the amines of proteins. There are numerous examples reported in the literature where the reaction of glutaraldehyde with a substituted primary amine results in the formation of products formed via a 1,4-dihydropyridine intermediate as depicted below in Figure 3 (Foos et al, 1979; Guerrier et al, 1983; Bonin et al, 1992; Yue et al, 1996; Burm et al, 1998; Katritzky, 1998).

Dihydropyridines are useful intermediates in the synthesis of pyridine containing compounds and play an important role in biological systems (Kosower and Sorenson, 1962; Eisner and Kuthan, 1972). The usefulness of the dihydropyridine as a synthetic intermediate, and as the proposed intermediate responsible for glutaraldehyde's biocidal activity, arises from its reactivity with both nucleophiles and electrophiles. From a biocidal point of view, those nucleophiles that would be present in an aqueous microbial environment include the hydroxyl groups of the amino acid serine, thiols groups of the amino acid cysteine, carboxylates groups of the amino acid aspartate, and amines groups of other lysine and arginine residues. Electrophiles in an aqueous environment most likely would include protons from water.

By extending these examples from the chemical literature, a theory on the mechanism of action of glutaraldehyde would go as follows. The initial reaction of glutaraldehyde with a lysine or arginine residue that is associated with either the microbial cell wall or of an essential protein associated with the cell wall would result in the formation of a reactive dihydropyridine intermediate. This unstable compound would then be capable of reacting with other nearby nucleophilic amino acids. These series of reactions would in effect crosslink the amino acid residues and would prevent the protein (or cell wall) from changing its tertiary structure in the manner that is required for it to function properly. Losing the ability to change its tertiary structure disrupts the normal functioning of the protein or cell wall and the protein ceases to work. This eventually results in cell death.

Compatibility concerns. Glutaraldehyde will react with, and be deactivated by ammonia, primary, and secondary amines (if present at high concentrations in alkaline systems). It is more stable at acidic pH's than at alkaline pH's, although its efficacy is faster at alkaline pH's. Glutaraldehyde will act as a biocide at both ends of the pH spectrum, but the optimum pH range for glutaraldehyde use is 7–10, as this pH range balances the residual stability of glutaraldehyde with its rate of efficacy. Glutaraldehyde will react with bisulfite-based oxygen scavengers to form an aldehyde-bisulfite addition product. This reaction is reversible and under high temperature

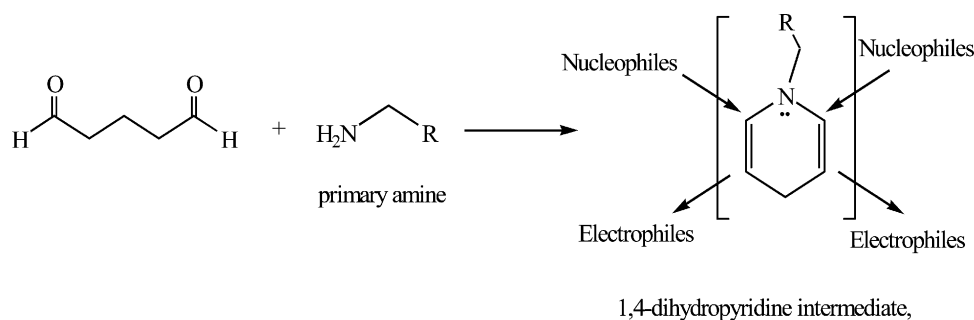
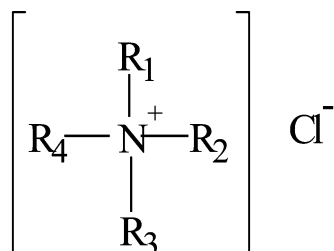


Figure 3 The reaction of glutaraldehyde with a primary amine.

conditions ($> 60^{\circ}\text{C}$), the complex will break down to release the glutaraldehyde. The reaction of glutaraldehyde with amines or ammonium ions is not reversible and can result in a product with little to no biocidal efficacy.

5.4.4.6 Quaternary ammonium compounds (QACs) [II, 18.1.]



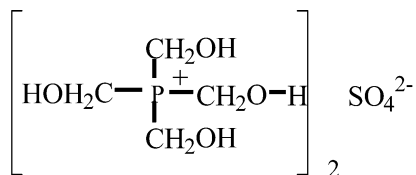
Where R_1 = alkyl (C_{14} - C_{18}) and R_2 , R_3 , and R_4 = methyl or benzyl
or R_1 and R_2 = alkyl (C_{10}) and R_3 and R_4 = methyl

General information. QACs have been used extensively in oilfield applications. They are positively charged molecules that consist of four alkyl groups attached to a central nitrogen atom. One of more of the alkyl groups attached to the central nitrogen usually will consist of methyl, benzyl, decyl (C_{10}), coco-(C_{14}), or soya (C_{18}) groups. QACs are commonly used in combination with other biocides and are usually inexpensive. QACs are also widely used as filming amine based corrosion inhibitors.

Mechanism of action. QACs have surfactant properties and therefore help to solubilize the cell membrane of microbial cells (Merianos, 1991). This leads to cell damage and ultimately cell death. QACs are especially effective when used in combination with other biocides that attack the cell wall. The surfactant action exhibited by the QAC helps to make the cell wall of microbial cells more accessible to other biocides. The QACs facilitate penetration of biocides such as glutaraldehyde, formaldehyde, and THPS and leads to enhanced biocidal activity of these biocides.

Compatibility concerns. Foaming is usually the biggest concern in using QACs as microbicides. Alkyldimethylbenzyl ammonium chloride [II, 18.1.2.] foams extensively while low foaming quats such as didecyldimethyl ammonium chloride [II, 18.1.4.] are now available. Foam formation is usually problematic in seawater systems where the foam induced by the QAC may interfere with the operation of the deaeration tower. The surfactancy of the QACs may also inhibit the separation of the oil/water emulsion in oilfield production systems. The salinity of oilfield water may impact the microbicidal activity of a QAC as high calcium brines may cause QACs to precipitate out of solution. Hard water is also said to decrease the antimicrobial activity of QACs (Petrocci, 1974). Lastly, the positive charge of the QACs can lead to negative interaction with other water additives such as negatively charged scale and corrosion inhibitors.

5.4.4.7 THPS [II, 3.6.]



Tetrakis(hydroxymethyl) Phosphonium Sulfate (THPS)
CAS # 55566-30-8

General information. Chemically, THPS is tetrakis(hydroxymethyl) phosphonium sulfate. It is used in oilfield applications, but has also been used in traditional water treatment applications as well as some non-biocidal applications. A closely related product, THPC (tetrakis(hydroxymethyl) phosphonium chloride) is a widely used flame retardant. THPS is used on both the injection and production side of an oilfield and has a unique mechanism of action which enables it to perform three functions in an oilfield. THPS will kill microorganisms, reduce hydrogen sulfide concentrations and react with and dissolve iron sulfide.

Mechanism of action. The actual active agent species is not THPS (the charged phosphonium salt) but is instead the uncharged trihydroxymethyl phosphine that is formed upon exposure of the THPS to a base. The reaction occurs because the β -protons of 1-hydroxyalkylphosphonium salts are actually slightly acidic and can be abstracted by a base (Hellman and Shumacher, 1960; Kirby and Warren, 1967). In aqueous systems, the base is hydroxide ion. The result of this proton abstraction is the generation of 2 moles of formaldehyde and 2 moles of the active biocidal species, trihydroxymethyl phosphine (THP) and is shown in Figure 4. This reaction, the cleavage of 1-hydroxyalkylphosphonium salts by alkali to give formaldehyde and the phosphine has been known since the early 1960's (Trippett, 1961).

The release of formaldehyde during the generation of the biocidally active phosphine is responsible for the reduction in hydrogen sulfide concentrations that have been observed during its use (Larsen et al, 2000). The release of formaldehyde probably is not responsible for any appreciable amount of biocidal action. In oilfield systems that have insoluble iron sulfide present, the use of THPS has been seen to help dissolve and remediate the problems associated with iron sulfide fouling (Walker, 1991; Diaz, 1998; Nasr-El-Din and Al-Humaidan, 2001; Jeffery et al, 2000; Gilbert et al, 2002). The species that is responsible for this reaction is actually the THP (trihydroxymethyl phosphine).

It is known from the organic chemistry literature that organic phosphines are excellent reagents for the reduction of disulfide bonds (Parker and Kharasch, 1959; Overman and O'Connor, 1976; Ruegg and Rudinger, 1977; Kirley, 1989). The biocidal activity of THP arises from the fact that it will react with the disulfide amino acids of a microbial cell wall (the cystine residues) and cleave the sulfur-sulfur bond. The cystine amino acid residues will be converted to cysteine groups and the phosphine will be converted to the phosphine oxide. These series of reactions are shown in Figure 5 below. The reduction of the disulfide bonds of the cystine residues destroys the integrity and tertiary structure of the cell wall and those proteins associated with the cell wall and ultimately results in cell death.

It should be noted that the chemistry of the THPS molecule is driven by the electrochemistry of the phosphine species. Phosphines exist in the +3 oxidation state and most phosphine chemistry results in a phosphorus +5 species. Such is the case for the mechanism proposed above.

Compatibility concerns. THPS is not compatible with oxidizing biocides, but is compatible with most other commonly used nonoxidizing oilfield microbicides such as QACs or glutaraldehyde. THPS is more stable under anaerobic conditions than aerobic conditions. This is due to its tendency of the THP molecule to be oxidized to the phosphine oxide in the presence of oxygen. It works faster in alkaline systems than acidic systems, since the active THP is formed faster under alkaline pH's. In anaerobic oilfields, THPS is extremely stable and has been observed to travel through a formation and reemerge in the produced water (Bryan et al, 1995). Also, being the sulfate salt, solutions of THPS have been observed to react with divalent ions such as calcium and barium in oilfield produced waters to form insoluble calcium sulfate and barium sulfate precipitates.

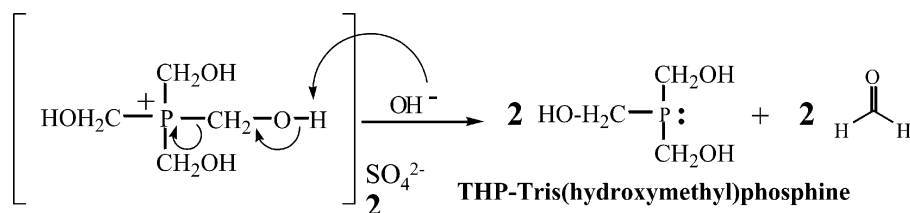


Figure 4 The formation of tris(hydroxymethyl) phosphine and formaldehyde.

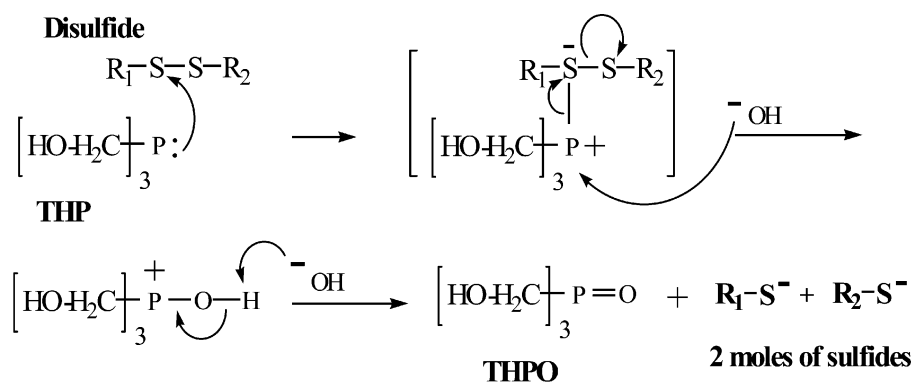
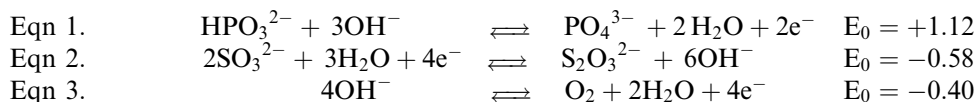
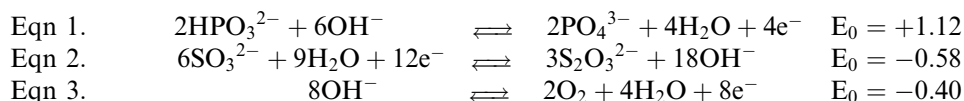


Figure 5 The reduction of an alkyl disulfide with tris(hydroxymethyl) phosphine.

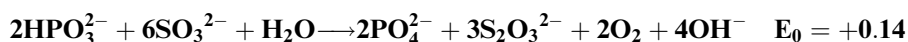
THPS, like many oilfield biocides, will react with chemical oxygen scavengers and will be deactivated. However, the reaction of THPS with bisulfite in aqueous systems may result in the generation of dissolved oxygen. This phenomenon is the result of a series of electrochemical reactions that is driven by the oxidation of the THP (which exists in the +3 oxidation state) to the phosphine oxide (which exists in the +5 oxidation state). Concurrent with the 2-electron oxidation of the phosphine is the reduction of bisulfite (or sulfite) to thiosulfate and hydroxide ion to molecular oxygen. The electrochemical half reactions, along with the standard reduction potentials under basic conditions for this series of half reactions are shown below. The E^0 values were obtained at the website entitled WebElements, operated by the Chemistry Department of the University Sheffield. The address for the redox potentials of phosphorus is <http://www.shef.ac.uk/chemistry/web-elements/P/redn.html>. The website address for the redox potentials of sulfur is <http://www.shef.ac.uk/chemistry/web-elements/S/redn.html>. The website address for the redox potentials of oxygen is <http://www.shef.ac.uk/chemistry/web-elements/O/redn.html>. (These redox potentials were adapted mostly from Bard et al., 1985).



The balanced half reactions are shown below.



The overall reaction and net E_0 values are shown below.



The Gibbs Free Energy of the overall process is calculated by using the following equation:

$$\Delta G = -n\mathbf{f}E_0$$

where $\mathbf{n}$ is the number of electrons, $\mathbf{f}$ is faraday's constant, and E_0 from above.

$$\begin{aligned} \text{So:} \quad \Delta G &= -(12)(23,060\text{cal/volt})(0.14)\text{volt} \\ &= -38740\text{ cal} \\ &= -38.7\text{ kcal} \end{aligned}$$

A negative ΔG means that this reaction should be spontaneous at basic pH. This calculation provides additional support for the experimental observation that oxygen is evolved when ammonium bisulfite and THPS are present under alkaline conditions. It is interesting to note that at acidic pH's, this reaction should not occur. Due to the concerns over oxygen induced corrosion of metal surfaces in oilfield systems, dissolved oxygen concentrations should be monitored if THPS will be used in waters that contain bisulfite.

5.4.5 The role of biocides in oil and gas operations

Biocides are used in oilfields for primarily two reasons; to preserve water-based fluids that are used in various oilfield operations, and to control biofouling. The fluids that are used in oilfield operations are varied in composition and properties, but require the use of a biocide to prevent the degradation of important components of the fluids. Biofouling control, in this case, refers to controlling the growth or proliferation of microbes that can potentially be harmful to the operation of the oilfield, or to the value of the hydrocarbon product that is produced. Both applications for biocides have in common the goal to limit or control the introduction of bacteria into the oil-bearing formation.

Fluids preservation in oilfield operations

There are many types of fluids used in oilfield operations. One thing that many have in common is that they are water based and as such require the use of a biocide to control the growth of microorganisms that may degrade the critical components of the fluid. Each type of fluid has its own set of characteristics and its own unique requirements for a biocide. The role of a biocide in the following fluids is to both preserve the integrity of the fluid and to prevent the introduction of potentially harmful microorganisms into the oil and gas bearing formation. Several types of fluid that require preservation, their components and their role in oilfield operations are as follow. The interested reader can refer to the Kirk Othmer Encyclopedia of Chemical Technology, Online version found at www.mrw.interscience.wiley.com/kirk/index.html for descriptions of these fluids.

5.4.5.1 *Water based drilling muds (Darley and Gray, 1998)*

During the drilling of a well, a drilling fluid is a vital component of the drilling process as it serves to:

1. Suspend the rock cuttings and carry them to the surface.
2. Lubricate and cool the bit and the drill pipe.
3. Seal the pores of the rock formation in order to prevent the in-flow of oil, water or gas.
4. Compensate for the reservoir pressure by means of hydrostatic pressure.

In order for the drilling mud to efficiently remove the rock cuttings from the well, it must be high in viscosity and density. A water based drilling mud typically consists of water (either fresh water or salt water), a chemical to increase viscosity (usually a form of clay but a polymer may also be used), and an inorganic chemical to increase density (usually barite). The most common form of clay that is used to increase viscosity is bentonite. However, some organic polymers may be used and they can be either polysaccharides (starch, guar or xanthan gum), cellulose (cellulose, lignosulfonates) or synthetic polymers (polyacrylamide). The most common inorganic chemical used to increase density is barite, or barium sulfate. Other inorganics such as sodium and calcium chloride may also be added to alter the properties of the mud. In addition to helping remove rock cuttings from the well bore, the high density of the drilling mud results in a very high hydrostatic pressure exerted by the column of drilling fluid in the wellbore. The weight of the column of drilling mud balances the downhole formation pressures and prevents the uncontrolled inflow of formation fluids that could result in a blowout.

Drilling muds are generally mixed on-site before injection into the well with whatever surface water is available. The surface water brings with it all of the contaminants normally found in surface water as well as a high concentration of dissolved oxygen. Dissolved oxygen in the water and in the mud can contribute to corrosion of the metal piping in the well. Maintaining a high pH is one means of corrosion control. Oxygen corrosion is reduced significantly at pH's of greater than 11 and is generally lower at any alkaline pH than in an acidic environment. As a result, many drilling muds are used at high pH's, although care is taken to ensure that the pH of the mud does not adversely affect the stability of the other components. It should be noted that drilling conditions dictate the composition of the drilling mud and it is left to the skill of the drilling engineer to formulate the mud according to those unique conditions.

Preservation of a drilling mud. A biocide is used in drilling muds primarily to prevent the degradation of the drilling mud, and also to prevent the introduction of potentially harmful bacteria into the formation. Micro-organisms in the water used to prepare the mud, or enzymes secreted by the microbes, can degrade the polymers used in the mud resulting in loss of viscosity and adverse rheological affects. The loss of viscosity could lead to a blowout that could cause serious injuries to the oilfield personnel working on the drilling rig. Once the viscosity is lost, the mud must be discarded and replaced with fresh mud. This is both time consuming and expensive. While the cost of the biocide is a minor part of the cost of formulating a drilling mud, its use is crucial to ensure the safe and effective use of the mud. There are several requirements for a drilling mud biocide. It is preferred that the active agent be fast acting but also has long-term preservative properties, be stable and effective at high pH's, and compatible with the other components of the mud. The most commonly used biocides for the preservation of drilling muds are glutaraldehyde and isothiazolones. For those drilling muds that are formulated at pH of 8 or lower, biocides such as DBNPA and bronopol may also be effective. Formaldehyde and formaldehyde release agents are generally being phased out of use due to concerns about health effects to the oilfield workers from formaldehyde exposure. Phenolics and quaternary ammonium compounds are also rarely used due to compatibility and/or worker exposure concerns.

5.4.5.2 *Completion and workover fluids (Darley and Gray, 1988)*

Completion and workover fluids are generally grouped together into the same category since they perform similar roles and contain similar components. Completion fluids are fluids that are used at the time that the drilling of the well has been completed and the well is in a transition process from drilling to production. These fluids are used mainly to counter balance the pressure in the formation, but they also can be used to clean out any remaining drilling muds from the downhole well bore area. The fluids generally consist of brines prepared from the chloride, bromide, and formate salts of sodium and potassium, and also the chloride and bromide salts of calcium, and zinc bromide. They may also contain organic polymers to increase the viscosity of the fluid. Among the many different types of polymers used are cellulose-based polymer such as hydroxyethyl- or carboxymethyl-cellulose or a modified xanthan gum. The density of the brine is adjusted by the choice of salt that is used.

Workover fluids are used during the time that a well is taken off-line and some mechanical operation is being performed on the well. The role of the fluid is to control the downhole pressure while the mechanical operation is being performed. Workover fluids contain brines that are similar to those described above and also may or may not contain organic polymers.

Preservation of a completion or workover fluid. The role of the biocide in a completion or workover fluid is much like its role in the preservation of drilling mud. It must protect the organic polymer component of the completion fluid from degradation, and prevent the introduction of potentially harmful bacteria into the formation. The biocide must be tolerant of high salt concentrations and it is preferable that it be both fast acting and have long term preservative properties. Glutaraldehyde is generally the preferred biocide for use in completion and workover fluids.

5.4.5.3 *Fracturing fluids (Bradley, 1987)*

During the normal course of operations, the volume of oil and gas produced from a well may gradually decrease. There are usually many causes of this but sometimes it is due to insoluble particulates plugging the pores of the formation. To help increase the production from the well, a stimulation procedure may be performed. There are several different types of stimulation's that may be performed and the one that is performed depends on what is causing the plugging of the formation. For example, if a carbonate scale is found to be the cause of the plugging problem, an acidizing treatment may be used in a procedure generally called a chemical stimulation. This operation involves pumping a solution of hydrochloric acid into the formation where it can dissolve the scale. This procedure can be difficult and extremely corrosive due to the large quantities of acid required. Another stimulation procedure is called formation fracturing. This procedure involves the pumping of a fracturing fluid into the formation under high pressure to induce fractures in the rock formation. The fracturing fluid is a heterogeneous solution of water, sand, and an organic polymer and its role is to carry the sand into the newly formed fissures and prop them open. The polymer serves to increase the viscosity of the fluid so that it can efficiently carry the sand into the formation. The polymers that are used include guar and derivatives of guar (hydroxypropyl guar).

Preservation of fracturing fluids. Fracturing fluids are often mixed on-site with surface waters so the role of the biocide is to protect the polymer in the fracturing from degradation, and to prevent the introduction of bacteria into the formation. The biocide is usually added into the water prior to the introduction of the other components of the fracturing fluid, so the preferred attributes of the biocide are fast kill but also long-term preservation. Also, due to the logistics of treating fracturing fluids, oilfield service companies are tending to favor biocides that are available in a solid form, or an easy to handle liquid form. As such, the biocides that are commonly used to treat fracturing fluids include DBNPA and bronopol as solid products, and THPS in smaller liquid quantities. Formaldehyde and formaldehyde release agents are not as popular as the other biocides listed above for this application.

5.4.5.4 *Packer fluids*

During the drilling of a well, a packer is often placed between the casing and the tubing. The casing is the outermost piping in a well and is often in contact with the rock formation itself. The tubing is set inside of the casing and the produced fluids arise from the formation within the casing. The space between the casing and the tubing is the annulus. The packer helps to stabilize the tubing within the casing and also helps to control and isolate the downhole pressures. As an additional safety measure, a packer fluid is often added into the annulus to help control the formation pressures. Without a packer fluid, the topside wellhead equipment may be exposed to the full pressure of the reservoir. There are both oil- and water-based packer fluids and the choice of which to use depends on the conditions of the individual well. Packer fluids may be left in place for varying lengths of time, and could be in place for years. As such, there are several important requirements for a packer fluid. It must be both mechanically and chemically stable under the conditions that it will be exposed to, should not cause corrosion, and should not have the potential to cause damage to the formation should it be exposed to it. Water based packer fluids will usually contain brines to increase density for pressure control. The density of the packer fluid can be adjusted by careful selection of the salts used to formulate it and that choice is dictated by corrosion potential, formation pressure, and cost. As with completion and workover fluids, the chloride and bromide salts are commonly used. In some fluids, a viscosifying agent may be used to control both pressure and to prevent fluid loss to the formation. These are usually organic polymers such as xanthan gums, lignosulfonates, or inorganic clays. Corrosion inhibitors may also be used to ensure that the fluid does not contribute to the degradation of either the casing or the tubing.

Preservation of a water-based packer fluid. The role of the biocide in a packer fluid is to prevent the degradation of the organic polymer and to prevent the growth of potentially harmful microorganisms once the fluid is in the annular space. The biocide should have long lasting preservative qualities, be compatible with the other components of the fluid, and be tolerate of the conditions it will see once the fluid is in-place. Commonly used biocides include quaternary ammonium compounds, glutaraldehyde and isothiazolone.

5.4.5.5 Hydrotest fluids

Upon the completion of the construction of, or repairs to, a pipeline, the integrity of the welds must be tested. This is usually done by filling the pipeline with water and pressurizing the pipeline to pressures that are beyond normal operating procedure. The water that is used will be any available surface water. Upon completion of the hydrotest, the water is usually kept in place until the pipeline is brought back into service. If the pipeline operator does not want to store the water in the pipeline, the hydrotest water is pump out of the pipeline and the pipeline dehydrated by the use of methanol. The hydrotest water is often disposed of back into its source (the sea or a river) but occasionally is pumped into the ground via a disposal well. Some hydrotests may call for the use of corrosion inhibitors and oxygen scavengers, as well as biocides, to decrease the potential for corrosion to occur during the test.

Preservation of hydrotest fluids. The use of biocides in hydrotests presents some unique challenges to the oilfield service companies. They have to balance the need to protect the pipeline from microbial attack, while also being concerned about the potential of negative environmental effects that may arise for a spill or discharge of biocide-containing water. The choice of a hydrotest biocide depends mainly upon the length of time the water will be kept in the pipe, the environmental fate profile of the biocide, and also whether the biocide can be easily deactivated. The most commonly used biocides in hydrotests are glutaraldehyde, THPS, and quaternary ammonium compounds.

5.4.6 Biofouling control

The other major application for biocides in the oilfield is in biofouling control. This can be defined as the use of a biocide to kill microorganisms that are present in the water that is either injected into an oilfield, or the water that is produced from the oilfield. The reason that biocides are used is to kill the microorganisms before they have the opportunity to attach to the metal surfaces and form biofilms. Considerations on the use of biocides in the injection and production systems are discussed below.

5.4.6.1 Water injection and production systems

As explained above, the injection system is responsible for pumping water into the formation for the purpose of forcing the oil and gas out of the formation and into the production wells. The production system is responsible for separating the produced fluids into their respective components of oil, water, and gas. Biocides are used on both sides of an oilfield in an effort to control:

1. Microbiologically Influenced Corrosion (MIC) of the injection and production system.
2. Hydrogen sulfide production due to sulfate-reducing bacteria growth and proliferation.

Control of MIC. Microorganisms in the injection water and produced water may have the opportunity to attach to metal surfaces in regions of low flow rates. If allowed to form biofilms, the microorganisms could contribute to microbiologically influenced corrosion (MIC) of the injection system. MIC events in the injection system can lead to corrosion failures of pipelines and pumps, with the related problems of equipment downtime, lost production and environmental hazards caused by spills or releases. It is safe to say that the overwhelming purpose of using biocides in the oilfield is to control MIC.

Most injection water biocides are added via slug doses as opposed to addition on a continuous basis. The advantage of slug dosing of biocide is that high concentrations are added for shorter periods of time. This method of treatment is usually more efficacious than treating with lower doses for longer periods of time (Grobe et al, 2002). However, there may be situations where a continuous feed of biocide, in which the entire volume of water is being treated, provides a more effective solution. In the production system, MIC events may be more common than in the injection system. The separators may contain large quantities of both water and solid deposits. Very often the flow through the separation system is not turbulent (since the separation process requires time to allow the oil/water emulsions to separate) and this could allow microorganisms the opportunity to attach to the available surfaces. The solid deposits present in the bottom of the separators also may also provide microorganisms with protection from biocide treatment. Both continuous and slug dose treatments of the production system are used, but the degree of success depends on how well the biocide can mix in the system and treat the affected surfaces.

Biocides for injection systems. The most commonly used biocides in injection systems include glutaraldehyde, THPS, quaternary ammonium compounds, acrolein, and formaldehyde. These biocides may be used alone, or in combination with each other. Blends containing glutaraldehyde, quaternary ammonium compounds, both

with and without formaldehyde are commonly used, as are blends of THPS and quaternary ammonium compounds with and without formaldehyde. Service companies blend these products to reduce cost, but also to reduce the likelihood that microorganisms develop tolerance to any one biocide. When mixing biocides, it makes sense to mix biocide modes of action. Hence, using a quaternary ammonium compound with either glutaraldehyde or THPS makes sense since the quat helps the other biocide penetrate the cell membrane of the microorganisms and react with the cell wall. It should be noted that regulatory requirements must be followed if multiple biocides are to be formulated together. Biocides can be co-fed from their respective containers into the system usually with no risk of violating any regulations. Lastly, if multiple biocides are going to be used at the same time, it is imperative that they be chemically compatible with each other. Blends of a primary amine type biocide (a cocodiamine for example) would not be compatible with an aldehyde biocide like acrolein or glutaraldehyde and so should not be used together.

Biocides for production systems. The most commonly used biocides for production systems include glutaraldehyde, THPS, formaldehyde, and acrolein. Quaternary ammonium compounds are used sparingly since their surface-active properties may interfere with the separation of the oil and water. Acrolein is often used because it exhibits biocidal and hydrogen sulfide scavenging properties. In addition, acrolein is one of the few products available that will remove hydrogen sulfide from the oil phase and the water phase, rather than just the water phase. Acrolein has also been used to dissolve iron sulfide. THPS use in production systems has been increasing due to its unique ability to dissolve iron sulfide. As noted above, iron sulfide in the production system can cause problems in the separation systems and the use of THPS has been shown to dissolve iron sulfide and improve the quality of the water in the production system. In addition, THPS can reduce hydrogen sulfide concentrations since it releases formaldehyde to generate the active phosphine species, and it is also an effective biocide. It should be noted that all of these biocides are effective products, but their efficacy will be limited if the production system is particularly dirty, or has solid deposits that will limit or hinder the ability of the biocide to react with the microorganisms.

Souring or H₂S control. Although some oil and gas fields contain hydrogen sulfide from the moment that they start production, most become sour over time (Khatib and Salinitro, 1997). This is due to the infiltration of bacteria into some portion of the field, and the proliferation of SRB's that then produce H₂S. It is hoped that the routine use of biocides both on the injection and production side will control the bacteria and thus prevent souring. However, once SRB's gain a foothold in the system, they are very difficult to control. Besides regular injection of the biocide, service and oil companies often employ other techniques to control souring. Most involve the cleaning of surfaces, tanks, etc to remove and eliminate the biofilms that harbor the problematic bacteria. Several of these operations are discussed below.

Pigging operations. Pipelines, especially those that carry water, are often the hosts of biofilm growth. The growth of SRB's within these biofilms can cause pitting type corrosion on the inner surfaces of the pipe, and lead to pipeline failures. To reduce the likelihood that the pipeline may host a biofilm, its interior surfaces can be cleaned by performing a pigging operation. A pig is a device that fits inside the pipeline and contains brushes and scrapers that are designed to clean the inner surfaces of the pipe. The pig is inserted into the pipe via a pig launcher, and is pushed along the pipeline by the force of the flowing liquids in the pipe. It scrapes and brushes the debris off the surfaces and pushes it ahead of it as it travels through the pipeline. The pig and debris are then removed at the pig catcher. Biocides are often used in pigging operations since this is a very efficient way of treating the freshly exposed surfaces of the pipe with a fairly high concentration of biocide (Patton, 1991). A slug of biocide is added into the water behind the pig and gets pushed through the pipeline by the force of the water behind it. Regular pigging operations using biocide can ensure that the pipeline stays free of biofilm contamination. Biocides used in pigging operations must be fast acting and not be deactivated by hydrogen sulfide. Glutaraldehyde, quaternary ammonium compounds, and THPS, either alone or in blends, are commonly used in pigging operations.

Sand jetting operations. As mentioned above, certain tanks in the separation system may often contain large quantities of solids. These solids can provide a refuge for microorganisms and make it difficult to eradicate them. Sand jetting operations are performed to stir up the solid deposits in the bottom of the tank and dislodge any microbes that may have been attached to these solid deposits. A sand jetting procedure is performed by injecting water into the bottom of the separation tanks at a high pressure to ensure that the solids are stirred up. Biocides are often added into the sand jet water as a means to kill any microbes that are dislodged during this procedure. These procedures are an effective means of controlling bacteria in the separators between the times that the tanks are physically cleaned. Glutaraldehyde is used in sand jetting operations, but any other fast kill biocide (DBNPA, THPS) should also be well suited for this application.

5.4.7 Alternatives to biocide treatments to control souring

There are efforts underway in the oilfield to find chemicals or treatment regimens that can serve as alternatives to biocides in an effort to control souring, or the effects of souring. Several of these methods are chemical treatments, while the others are physical treatments.

The use of nitrate and nitrite

The most promising of the nontraditional treatments is the use of nitrate (Larsen, 2002; Thorstenson et al, 2002) and/or nitrite (Sturman et al, 1999) to reduce H_2S concentrations. This technology involves the continuous downhole injection of either nitrate or nitrite solutions to reduce the occurrence of souring. Several field evaluations of these chemicals have demonstrated that the continuous injection of nitrate or nitrite reduces the amount of H_2S that is produced from the field. The benefits of this technology are that nitrate and nitrite are not considered biocides and therefore do not require any special regulatory approvals and may be easier to handle than traditional biocides. There are however, some environmental concerns about the use of nitrate and nitrite, specifically the disposal of produced water that may contain residuals of each chemical.

The exact mechanism of how nitrate/nitrite treatments reduce H_2S concentrations is the subject of some debate. Nitrite is an oxidizing agent and is well known to oxidize hydrogen sulfide. However, the mechanism by which nitrate reduces souring is more complex. Voordouw (Gevertz et al, 2000; Nemati et al, 2001a; Nemati et al, 2001b; Nemati et al, 2001c) has described the isolation of *Thiomicrospira sp.*, strain CVO, which is referred to as a nitrate-reducing, sulfide-oxidizing bacterium (NR-SOB). This particular organism has been shown to oxidize sulfide to sulfur or sulfate, while reducing nitrate to nitrite, nitrous oxide or nitrogen. The mechanism by which the use of nitrate leads to decreases in souring is thought to involve two possibilities (Nemati et al, 2001b). The first is the introduction of nitrate and the stimulation of the growth of NR-SOB alters the environmental redox potentials to the point that they are inhibitory to SRB's. The second possibility is that in the presence of sufficient amounts of nitrate, the NR-SOB can directly oxidize sulfide, and remove it from their environment. The use of nitrate and nitrite to control souring is interesting and requires more study to determine its overall applicability in the reduction and control of souring.

Sulfate removal by reverse osmosis membranes

Several oilfields currently in operation or under construction in the North Sea, the West Africa coast, and Brazil are using sulfate-removal reverse osmosis membranes in an attempt to control or minimize reservoir souring (Davis and McElhinney, 2002; McElhinney and Davis, 2002). The theory behind the use of these membranes is that the removal of sulfate from the injection water will affect the metabolism of the sulfate reducing bacteria to the point that they can no longer produce hydrogen sulfide (Bakke et al, 1992). However, laboratory results demonstrated that it was not feasible to completely eliminate sulfide production. The research did suggest that removing sulfate may delay the production or appearance of sulfide, and may increase the amount of time the reservoir operates until it becomes sour. In actual use, the benefit found from the use of these membranes has been barite (barium sulfate) scaling control.

Irradiation

Irradiation of water with ultraviolet light has been used to control bacteria in seawater for injection into North Sea oil fields (Clark et al, 1984). For ultraviolet light to be effective, the water should be of low turbidity and free of slime, silt, suspended hydrocarbons and dissolved hydrocarbons (Patton, 1991).

The use of anthraquinone

Recently, the use of anthraquinone has been described as an inhibitor of those metabolic pathways in SRB's that reduce sulfate to sulfide (Harless et al, 2000). While not a biocide, anthraquinone is thought to be a biostat and its role is to inhibit or reduce the production of hydrogen sulfide. By definition, a biostat is a chemical that inhibits or interferes with specific enzymatic pathways without harming or killing the microorganism. The theory behind the use of biostatic compounds is their routine application can inhibit the critical processes involved in the formation of organic acids and hydrogen sulfide. Anthraquinone is one commercially available biostat, whose use has recently been described in oilfield operations (Burger et al, 1995; Johnson et al, 1999). The mechanism of action of anthraquinone is thought to involve the disruption, or inhibition, of the electron transfer process that is required for SRB to reduce sulfate to sulfide and thereby reduces biogenic hydrogen sulfide (Cooling III et al, 1996; Burger and Odom, 1999).

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5.5 A review of the microbiological degradation of fuel

J.A. ROBBINS and R. LEVY

5.5.1 Introduction

Fuel biofouling problems have been well documented since the middle of the Twentieth Century. Three areas of concern to the petroleum industry are microbial contamination in oil field injection water and kerosene and diesel fuel storage systems. Light fractions of fuel (mainly kerosene) and middle distillate fuel (predominately diesel fuel) are very susceptible to microbial contamination during storage in fuel tanks and in road vehicles, rail-cars, aircraft, generators, heating oil and marine vessels. The average fuel storage tank, which contains fuel and a water bottom, provides an ideal growth environment for microorganisms. In these systems, the microorganisms grow in the water phase of the fuel system, not in the fuel directly. The issue in oil field injection water applications, not covered in this chapter, is different. The injection water, used in the production of crude oil, must be protected against contamination by sulfate and thiosulfate reducing bacteria.

This chapter addresses the growth needs of microorganisms, the presence of microorganisms in fuel and the fuel storage distribution system, the consequences of microbial growth, tests to determine microbial growth and ways to prevent or eradicate these microorganisms.

5.5.2 Historical background

One of the earliest reports of microbial attack in fuels and oils was reported in the 1930's, when accelerated corrosion in aircraft fuel storage systems was attributed to sulfides produced by bacteria (Neihof, 1988). Bushnell and Haas (1941) worked with fuel water bottoms that were contaminated with microorganisms. They found that many of these microorganisms could utilize hydrocarbons as a carbon source. During World War II, gasoline fuel storage tanks in tropical areas experienced fuel "souring" caused by sulfate reducing bacteria (SRB) (Gardner, 1971). There were reports of microbial contamination problems in aviation gasoline in the early 1950's and in aviation kerosene in the late 1950's (Genner and Hill, 1981). It was recognized that more favorable conditions for microbial contamination existed in aviation fuels in 1960 with the switch from piston engines using gasoline type fuels to gas turbine engines using kerosene type fuels. The kerosene fuels were less toxic to microorganisms and contained more dissolved water, which is essential for microbial growth (Genner and Hill, 1981). Problems of filter plugging, localized corrosion and aluminum wing tank perforation were reported. Fungi were also identified as a problem in fuel contamination. *Hormoconis resinae* was found to metabolize hydrocarbons, form thick mats in the fuel storage tank and survive in the dry fuel for long periods of time (Neihof, 1988). The United States Air Force noted a decrease in microbial contamination problems when the anti-icing additive ethylene glycol monomethyl ether (EGME) was used in their JP-4 jet fuel in 1962 (Neihof, 1988). There have been complaints from military installations since 1965 of problems with diesel-powered equipment such as malfunctions, corrosion, filter plugging, and sludge formation (Rogers and Kaplan, 1982). Microbial contamination problems continued to be reported throughout the 1970's and 1980's with problems surfacing in marine vessels (Neihof and May, 1983) and diesel fuel for road vehicles (Reddy, 1988). Three hundred fifty samples from the handling systems of Jet A-1 aviation turbine fuel, collected from 1990 to 1996, were contaminated with predominantly *Hormoconis* and *Aspergillus* in the fuel and *Pseudomonas* spp., *Flavobacterium* and *Aeromonas* in the water bottoms (Ferrari et al., 1998). Sulfate reducing bacteria were isolated from 80% of these water samples.

5.5.3 Fuel distribution system

Fuel, manufactured from crude oil at company refineries, is transported to a main terminal through one of the major pipelines, by ocean tanker, by barge and/or by rail tank car. In the major pipeline, fuel is continuously pumped through the line and there is a mixing and merging of fuel of potentially different grades from different companies. At the main terminal, the fuel is delivered to a large, company-operated storage tank that can hold many different types of fuel, with the exception of aviation fuel. Aviation fuel must have its own dedicated tank. The fuel is sent from the main terminal storage tank by barge, truck, pipeline or rail to a local distribution tank. Then it is moved on to truck fleets, gas stations, airport storage and to the individual end-users (military, aviation, marine, utility companies and other commercial enterprises). Microorganisms can be introduced into the fuel at any of these various storage tanks or in the carriers that transport fuel between the tanks.

Table 1 General sizes of the fuel storage tanks in the fuel distribution system and the estimated fuel to water ratios

Storage tank	Size of tank		Amount of fuel		Estimated Fuel:Water ratio
	Diameter, meters (ft.)	Height, meters (ft.)	Gallons ^a (m ³)	Barrels ^b	
Strategic storage	unknown	unknown	unknown	unknown	unknown
Terminal tanks	up to 90 (300 ft.)	up to ≈12 (≈40 ft.)	50,000–20 million (200,000–75,000 m ³)	≈1,200–476,000	not available
Large tanks	up to 70 (230 ft.)	≈9 (≈30 ft.)	10 million maximum (38,000 m ³)	≈250,000	1,000:1–10,000:1
Fuel depot	20–40 (66–131 ft.)	12–15 (39–49 ft.)	> 20,000 (75 m ³)	> 475	500:1–5,000:1
Small tanks	3–3.5 (10–12 ft.)	≈6 (≈20 ft.)	2,000–12,000 (7–45 m ³)	≈200–290	100:1–500:1
Delivery tankers	–	–	7,000–9,000 (25–30 m ³)	≈167–214	–

(Information provided by Mr. John Miller, Rohm and Haas Company, Philadelphia, Pennsylvania, U.S. and Mr. Howard Chesneau, Fuel Quality Services, Inc., Flowery Branch, Georgia, U.S.)

^a1 U.S. gallon = 3.78 liters

^b1 barrel = 42 gallons (U.S. gallon)

Fuel storage tanks are located above ground or underground and range in size from the very large strategic tanks at the refineries to small tanks at service stations (see Table 1). Above ground storage tanks are vertical tanks with three different types of bottoms: flat, “cone up” or “cone down” (newer tanks). Some of these tanks have a center sump to remove water. The “cone up” bottom tanks may have a trench around the cone with sumps placed inside the trench. The terminal, large storage and fuel depot tanks are above-ground tanks. The ratio of the fuel to water in the fuel storage tank varies depending on the size of the tank, the type of tank bottom, age and condition of the tank, tank location (underground versus above ground) and the tank roof (floating versus fixed).

Because underground tanks are hidden from view, tank damage, such as corrosion and leakage, is hard to detect (Gaylarde et al., 1999). New U.S. regulations require that the underground tanks be protected against corrosion using such measures as protective coatings and/or cathodic protection, which involve the use of sacrificial anodes (Gaylarde et al., 1999). Service station and strategic storage tanks are examples of underground tanks. The strategic storage tank is designed with a tank liner and then a layer of concrete to insure tank integrity. The actual size of the strategic storage tank or the volume of fuel it holds may not be known because in some cases this “tank” is actually a cavern. Generally, the strategic storage tank holds crude oil in the United States and crude oil and finished product overseas. The level of fuel in these tanks is raised or lowered by pumping in water. The small service station tanks are horizontal tanks angled slightly downward to collect water. It is difficult to drain accumulated water from underground tanks, which makes them more prone to microbial contamination. Also, the prolonged storage of fuel in these tanks has led to an increase in microbial contamination (Smith, 1991).

5.5.4 Fuel types and their susceptibility to microbial contamination

Petroleum fuel

Crude oil (petra-oleum; two Latin words meaning rock and oil) is a complex mixture of liquid aliphatic, alicyclic and aromatic compounds and gaseous hydrocarbons with small quantities of nitrogen, oxygen, sulfur organic compound and traces of metallic components. The major classes of fuel are fractionated from crude oil at the refinery (Table 2). Using a continuous distillation process, crude oil is pumped through a distillation tower or column. Crude oil components separate at increasing temperatures. The lightest fractions with low boiling points and high volatility, such as propane and butane, come off first. They are followed by gas, gasoline, kerosene,

Table 2 The types of fuel products refined from crude oil

Fraction	Composition	Boiling point range, °C
Gasoline	Straight chain and branched-chain hydrocarbons, alkenes, naphthalene, aromatics, additives, chain length of C ₅ to C ₁₂ .	90–220
Virgin Naphtha (light distillate)	Mixed light hydrocarbons, product from the first distillation of crude oil or coal tar. Used as fuel and raw material in the manufacture of paints and varnishes. Component chain length of C ₅ .	150
Kerosene (middle distillate)	Paraffins. Chain length of C ₁₀ to C ₁₆ . Mixture of kerosene and gasoline fractions with an aromatic hydrocarbon content < 25%. For kerosene and aviation fuel.	120–200
Light gas oil (middle distillate)	Chain length of C ₁₅ to C ₂₂ . For #2 fuel oil, diesel fuel and jet fuel.	200–310
Heavy gas oil (heavy distillate)	Used as petrochemical feedstocks. Compounds with chain length ≈C ₂₅	up to 350

(Information from Satterfield, 1991; Gaylarde et al, 1999; Chevron, 2002)

diesel, and fuel oil. The heaviest fractions with high boiling points and low volatility, such as coal tar, separate last. The distillation process is followed by an upgrading process that removes undesirable trace components, such as sulfur and nitrogen compounds, by hydrotreating the fuel with hydrogen and a catalyst. A conversion process breaks down (cracks) large, high boiling point hydrocarbons left in the distillation column to smaller molecules in the middle distillate boiling point range. This is done by catalytic cracking, with high temperature plus catalysts, or by hydrocracking, which is similar to catalytic cracking with the addition of high-pressure hydrogen (Chevron, 2002).

Hydrocarbon-utilizing microorganisms are bacteria, molds or yeast that can use hydrocarbons as their sole carbon source for growth. Most hydrocarbon-utilizing microorganisms are capable of oxidizing fuel with carbon chain lengths of C_{10} to C_{20} . Therefore, kerosene (including aviation fuels) and the middle distillate fuels (diesel fuel contains alkanes of chain lengths of C_{12} to C_{21}) are the most prone to microbial attack.

Gasoline is a lighter fuel than kerosene. Gasoline has a low capacity for carrying dissolved water, which makes it less susceptible to microbial contamination than kerosene or the middle distillate fuels. Since 1974, changes in gasoline chemistry have made gasoline a more vulnerable target for microbial attack. In 1974, the EPA mandated that lead compounds (which have antimicrobial properties) should be phased out of gasoline. Also, certain states (i.e., California) and heavily populated metropolitan areas have mandated that oxygenates, either ethers (Ethyl Tertiary Butyl Ether, Methyl Tertiary Butyl Ether, Tertiary Amyl Ethyl Ether, or Tertiary Amyl Methyl Ether) or alcohols (ethyl or methyl alcohol) be added to gasoline to provide a cleaner fuel burn by reducing the carbon monoxide emissions. At very low concentrations, these alcohols and ethers could serve as a nutrient source for microorganisms, stimulating growth; however, at higher concentrations they reduce water availability and thus would have a negative impact on microbial growth (Hill and Koenig, 1995). In recent years, the demand for a higher yield of gasoline from crude oil led to fuel cracking to produce gasoline with a higher concentration of alkenes and a much lower concentration of aromatics including benzene (limited to 5%) (Hill and Koenig, 1995). The straight chain alkenes are easier for microorganisms to degrade than the aromatic compounds. Hill and Koenig (1995) recovered bacterial contamination from a gasoline tank in Western Europe. This study also suggested that this microorganism could use low levels of Methyl Tertiary Butyl Ether (MTBE) for growth. Passman *et al* (2001) found that 60% of approximately 400 refinery, terminal and retail outlet gasoline storage tanks, evaluated in 1992 to 1996, contained significant microbial contamination.

Aviation fuel is essentially kerosene, but has a separate designation because the industry requires strict specifications and a dedicated distribution system for this product. Civilian aviation fuel is classified as Jet A1 (most widely used), Jet A or Jet B by the American Society for Testing and Materials (ASTM). The Military and NATO have their own aviation fuel classifications. A typical aviation kerosene fuel contains the following mixture of hydrocarbons (Park, 1975): paraffins ($\approx 57\%$, straight chain C_8 to C_{19} alkanes, saturated C_nH_{2n+2}), naphthenes ($\approx 26\%$), aromatics ($\approx 17\%$), oleofins (1%), other cyclic compounds, mercaptans and performance additives (antistatic agents, dispersants/detergents, metal deactivators, stabilizers, etc.). This fuel is known to be susceptible to microbial contamination.

Middle distillate fuels are blended from light gas oil streams. Since 1977, changes in the refining of middle distillate fuel, with the use of a catalytic cracking process, have lead to increased microbial contamination. These fuels now contain more aromatic compounds and are more soluble and more readily emulsifiable in water (Smith, 1991). Middle distillate fuels include diesel fuel and furnace oil (which in many cases are the same and both are called #2 oil).

The ASTM Committee D-2 (Petroleum Products and Lubricants) is the committee responsible for diesel fuel specifications (Table 3) and test methods. ASTM D975 identifies five grades of diesel fuel (Low Sulfur No. 1-D, No. 1-D, Low Sulfur No. 2-D, No. 2-D, No. 4-D) and sets the limits of diesel fuel properties in the United States (ASTM, 2000a). The liquid fuels as defined by ASTM specifications D396, D910, D975, D1655, D2069, D2880, D4814 01a-D6227 are susceptible to microbial contamination.

Table 3 American Society for Testing and Materials specifications for fuel products

ASTM specification no.	Specifications for	ISO designation
D 396	Fuel oils	ISO 8217
D 910	Aviation gasoline	ISO 4261
D 975	Diesel fuel oils	
D 1655	Aviation turbine fuels (jet engines)	
D 1835	Liquefied petroleum	
D 2069	Marine fuel	
D 2880	Gas turbine fuel oils	
D 3699	Kerosene	
D 4814-01a	Automotive spark and engine fuel (gasoline)	
D 5797	Fuel methanol	
D 5798	Fuel ethanol	
D6227	Unleaded Aviation gasoline	

Synthetic fuels

We have become dependent on the use of petroleum fuels to provide heat, power equipment and provide transportation. This has led to a concern that there could be an interruption in a secure, adequate supply of crude oil needed to manufacture these fuels. Continued reports of microbial contamination in petroleum fuels have also caused concern. Scientists have been evaluating the use of alternate sources to petroleum to prepare fuel (synthetic fuels) such as oil shale, tar sands and coal. Studies of the microbial susceptibility of Jet JP-5 and diesel fuels prepared from these alternate sources demonstrated that the synthetic fuels would not support more microbial contamination than their petroleum-derived fuel counterparts and that fuel derived from pyrolyzed coal may even have fewer problems (May and Neihof, 1982). Coal JP-5 jet fuel inhibited growth of *Hormoconis resinae* and *Candida* spp. (caused by constituents in the coal-derived fuel that have not been identified) (Neihof and May, 1984).

Biodiesel fuels

Biodiesel fuels are also being investigated as an alternate source to petroleum fuels (Chevron, 2002). These fuels are mixtures of fatty acid methyl esters, which can be burned straight or utilized in blends with diesel fuel. Biodiesel fuels are prepared from vegetable oils (i.e., soybean oil) or animal fats and exhibit similar chemical and physical properties as petroleum prepared diesel fuels except that the biodiesel fuels contain no aromatics or sulfur. At the present time, the major disadvantages of these fuels are the relatively high cost and a high pour point that would limit their use in cold weather. Of more importance to microbiologists, these fuels are readily biodegradable and it is probable that they would be subject to increased microbial growth during storage. The preparation of the specification for biodiesel fuel is in progress and should be approved soon. ASTM does have a provisional specification (PS 121) for a biodiesel fuel blend that includes a low sulfur biodiesel, B100, in combination with Specification D975 grade diesel fuels (ASTM, 2000d).

5.5.5 Growth requirements for microorganisms

In reality, the fuel storage tank is a two-phase system – a fuel phase and a water bottom phase. Some bacterial cells and fungal spores can survive dormant in dry fuel for months to several years (*Hormoconis resinae*) (Gardner, 1971). However, cells can only grow and reproduce in the water phase, primarily at the fuel/water interface where all their growth requirements can be provided. Microorganisms require free water, an organic nutrient source for energy, inorganic nutrients and proper temperature and pH for growth. Some microorganisms require oxygen for growth, while other microorganisms grow in the absence of oxygen. All of these requirements are met in the fuel storage tank and in the distribution lines.

Water

Free water is a fertile growing environment for microorganisms. When fuel is first delivered to the fuel tank, there may be little or no free water present. Free water becomes available from rainwater (especially in storage tanks with “floating roof” tops), ship ballast water, water leaking through faulty tank seals and vents in the system, residue from tank cleaning and in the fuel delivery. Condensation also adds to the free water. As the fuel cools, water will condense and free water droplets will form on the sides and bottom of the tank. Water is heavier than fuel, so it generally falls to the bottom of the tank. As microorganisms start to grow, cellular metabolism produces more free water (water is an end product of hydrocarbon degradation). *Hormoconis resinae* can produce 0.94 g water per liter of fuel after four weeks (Hill and Thomas, 1975).

Dissolved water is also present in the fuel. The amount of water solubility in fuel is related to the hydrocarbon chain length, the presence of an aromatic structure, and temperature. Shorter chain paraffins dissolve more water than the longer chain paraffins. Kerosene fuels are more susceptible to microbial attack because they have a greater capacity to absorb dissolved water than other fuel types. There is 1 part per million (ppm) of dissolved water in aviation kerosene fuel for every degree Celsius (C) above zero (Park, 1975). An aromatic hydrocarbon can dissolve five times more water than straight chain hydrocarbons. Chemicals used to treat fuel such as preservatives can also contribute to the dissolved water content.

Hydrocarbons – organic nutrients

There are an abundance of nutrient sources available for microorganisms in the fuel storage tank. Hydrocarbons (80 to 89% carbon) (Hettige, 1993) serve as a carbon source for a wide variety of microorganisms. Microorganisms can metabolize straight chain aliphatic hydrocarbons and the lower molecular weight cyclic and aromatic molecules found in petroleum fuel for their energy production. Microorganisms start to degrade these fuel hydrocarbons at the same time, but at different rates of activity. Straight chain alkanes are degraded the most rapidly. The branched alkanes, cycloalkanes and aromatics are more slowly degraded (Bartha and Atlas, 1987).

The ease with which microorganisms can utilize hydrocarbons depends on the type of hydrocarbons present (Wyatt, 1984; Bartha and Atlas, 1987; Hettige, 1993) and the microorganisms (Table 4).

- Many different microorganisms can degrade and assimilate aliphatic hydrocarbons.
- Aliphatic and long chain hydrocarbons are more susceptible to microbial degradation than aromatic and short chain hydrocarbons.
- Straight chain hydrocarbons are easier to degrade than branched hydrocarbons.
- Saturated hydrocarbons degrade more quickly than unsaturated hydrocarbons.
- Microbes can partially oxidize aromatic hydrocarbons. Only a few bacteria can assimilate them.

Hydrocarbons are water insoluble. To utilize the hydrocarbons, the microorganisms must first bring them inside the cell. It has been demonstrated by electron microscopy that microorganisms, grown on alkanes, contain droplets of accumulated alkanes within the cells (Ratledge, 1988). Hydrocarbon uptake cannot occur with only direct cell contact (Smith, 1991). Although the exact mechanism of hydrocarbon uptake is not known, there is evidence supporting the following route of hydrocarbon passage into the cell (Smith, 1991). Extracellular biosurfactants emulsify fuel into water at the fuel/water bottom interface, increasing the exposure of the fuel to the microorganisms and their metabolites. Microorganisms produce fuel-solubilizing agents that combine with hydrocarbon molecules. These solubilizing agents, acting as a carrier, transport the hydrocarbon molecules into the aqueous phase surrounding the microorganism, through the cell wall and cytoplasmic membrane into the cell. The hydrocarbon is trapped as an intracytoplasmic inclusion inside the cell.

Microorganisms utilize aliphatic hydrocarbons in the following steps (Wyatt, 1984; Bartha and Atlas, 1987; Smith, 1991) (see Figure 1):

1. Kerosene fuel contains approximately 57% alkanes. Alkanes with 10 to 22 carbon unit chains are the most easily utilized hydrocarbons. These alkanes are oxidized via the enzyme monooxygenase to yield the corresponding alcohol. This oxidation can be terminal or subterminal. With an attack at the terminal carbon, the hydrocarbon is converted directly to the primary alcohol. Some microorganisms will degrade hydrocarbons at a subterminal carbon, converting the hydrocarbon first to a secondary alcohol that is then further oxidized to a ketone and ester (Wyatt, 1984; Bartha and Atlas, 1987). The ester is finally hydrolyzed to form an acid and the primary alcohol.
2. The alcohol is oxidized via the enzyme alcohol dehydrogenase to yield the corresponding aldehyde.
3. The aldehyde is oxidized via the enzyme aldehyde dehydrogenase to yield fatty acid, which can be further metabolized by the cell.
4. Starting with even number carbon chain alkanes: the fatty acid is oxidized by beta-oxidation to acetate. Acetate is then available for use in the Krebs cycle.
5. Starting with odd number carbon chain alkanes: the fatty acid is oxidized by beta-oxidation to propionate.

Beta-oxidation of the fatty acids by aerobic microorganisms is catalyzed by acyl-CoA synthetase to generate coenzyme A esters. Even number fatty acid chains form acetyl CoA and odd number chains form acetyl CoA and propionyl CoA.

Alkenes are attacked by microorganisms at the saturated terminal carbon, oxidizing the alkene to an epoxy compound then to a diol. One of the hydroxy groups of the diol is further oxidized to a carboxyl compound that is cleaved to form a fatty acid and a primary alcohol (Bartha and Atlas, 1987).

Table 4 A comparison of the biodegradability of petroleum fuel hydrocarbons

Hydrocarbons	Comments on utilization by microorganisms
	Aliphatic Hydrocarbons
Straight chain alkanes	C ₁ to C ₄ – Few species can utilize this group (require special enzymes). C ₅ to C ₉ – microbial membranes do not tolerate solvent character. Limited utilization by some bacteria and fungi. Not utilized by yeast.
Branched alkanes	C ₁₀ to C ₂₂ – Most readily biodegradable hydrocarbons. > C ₂₂ – low water solubility, generally solids.
Alkenes	Utilized less than straight chain alkanes. CH ₃ branching in the 3-position → resists β-oxidation
	Cycloalkanes
Low molecular weight cycloalkanes	Rarely utilized. Solvent properties are toxic to microbial membranes.
	Aromatic Hydrocarbons
monoaromatic	Solvent properties are toxic to microbial membranes; can be utilized at low concentrations.
condensed polyaromatics – 2 to 4 rings	Less toxic than the other aromatics.
condensed polyaromatics – 5 or > rings	Not utilized.

(Table prepared from information in Bartha and Atlas, 1987.)

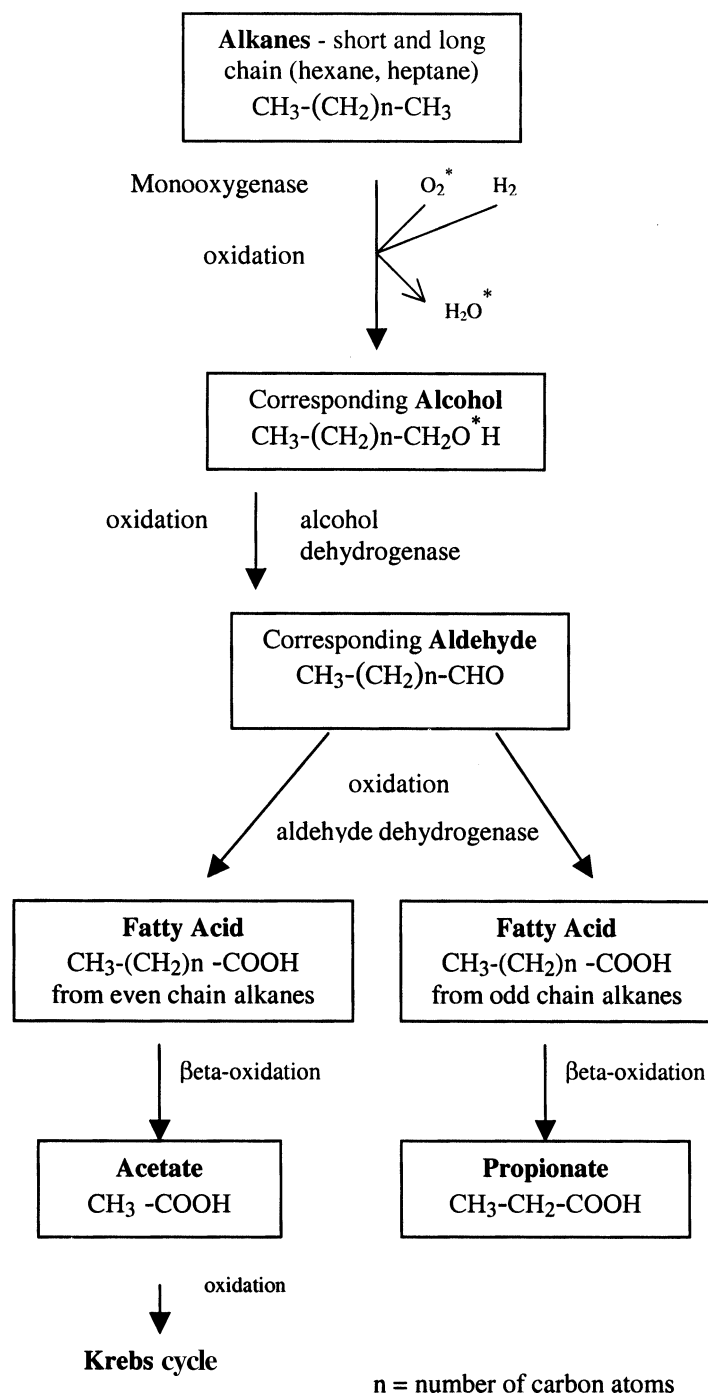


Figure 1 Oxidation of aliphatic hydrocarbons by microorganisms to form organic acids. (Information from Wyatt, 1984; Smith, 1991)

Aerobic microorganisms utilize lower molecular weight cyclic and aromatic hydrocarbons by first excising the side chains and oxidizing them to form fatty acids (Smith, 1991). Then microorganisms, via oxygenase enzyme activity, incorporate two molecules of oxygen onto the aromatic nucleus to form a dihydrodiol. The dihydrodiol is then oxidized to form a catechol. The catechol ring is opened by either an ortho or meta cleavage to eventually form a diterminal acid or acid aldehyde (Bartha and Atlas, 1987; Smith, 1991).

Oxygen

Oxygen is used by aerobic microorganisms to generate energy for growth. Obligate aerobic microorganisms require oxygen for respiration and biosynthesis. Facultative aerobic microorganisms, such as *Escherichia coli*, may grow aerobically in the presence of oxygen or fermentatively in the absence of oxygen. They do not use oxygen for biosynthesis. Microaerophilic microorganisms require low concentrations (less than 0.2 atm.) of oxygen for respiration (Brock et al., 1984). However, if the oxygen level is higher than 0.2 atm., it could have a toxic effect on these microorganisms. Microorganisms such as *Pseudomonas* utilize oxygen for aerobic

respiration, but may use nitrate for anaerobic respiration. Oxygen is available in the fuel storage tank dissolved in fuel, dissolved in the water bottom and present in the tank headspace. Kerosene fuel may contain > 300 ppm of dissolved oxygen (Genner and Hill, 1981).

Anaerobic microorganisms, such as sulfate reducing bacteria (SRB), are microorganisms that grow in the absence of oxygen. They are unable to generate energy by using oxygen as a terminal electron acceptor. SRB have been isolated from contaminated fuel tanks that were generally heavily fouled with microorganisms. Heavy contamination of aerobic microorganisms in the water bottoms can produce biomass formation with anaerobic conditions underneath. Also, oxygen can be depleted by aerobic microbial respiration creating anaerobic conditions in areas of the water bottom.

Inorganic nutrients

The major inorganic nutrients needed for microbial growth and metabolism include nitrogen, sulfur, phosphorus, potassium, magnesium, calcium and iron. Trace elements of cobalt, copper, manganese, molybdenum, selenium and zinc are also required by most microorganisms (Brock et al., 1984). Sodium chloride, tungsten and nickel may be needed by some microorganisms. These inorganic nutrients are available in tank sediment, water and dust. Phosphorus is considered to be one of the major growth limiting factors in fuel since it is present at less than 1 ppm (Gaylarde et al., 1999). Reportedly, fuel additives can provide these nutrients, such as nitrogen and phosphorus both from organic amines (Bento and Gaylarde, 1998) and nitrogen and sulfur from gum inhibitors (Park, 1975).

Temperature

Each microorganism has a range of minimum, optimal and maximum temperature that affects its growth and survival. As the temperature increases within this range, the metabolism of the microorganism increases (Brock et al., 1984). Above the maximum temperature, cellular metabolism ceases to function and the microorganism dies. The optimal temperature for the growth of most fuel microorganisms is 25° C to 30°C. The average moderate temperature in the fuel tank is 20° to 30°C. However, microbial growth has been reported in fuel with temperatures ranging from -2°C to 55°C (Genner and Hill, 1981).

Effect of pH

Microbial growth has been discovered at extreme pH levels of < 1.0 for acidophiles to 13.0 for alkalophiles. In general, the majority of bacteria prefer a neutral pH. Fungi prefer slightly acidic conditions (pH 4-6) for growth and SRB grow best at pH 7.5 (range of growth is pH 5 to pH 9) (Hill, 2000). The pH of a fuel storage tank water bottom is generally between 6 and 9, so pH should not limit the ability of most microorganisms to grow in this environment (ASTM, 2001). Seawater, used as ballast in marine vessels, has a pH of approximately 8.

The sample pH can have an affect on the types of microorganisms that are present. Neihof and May (1983) studied the types of microorganisms present in sludge from more than eighty fuel tanks on eight naval vessels where seawater replaced fuel for ballast. In this study, *Hormoconis resinae* was present only when the yeast *Candida* sp. was present. *H. resinae* does not grow well at the slight alkaline pH of seawater. *Candida* produces acidic metabolites that may lower the pH of the seawater in the tank enough to allow *H. resinae* to grow (May and Neihof, 1981). These studies also demonstrated that a low pH inhibited bacteria and a high pH inhibited fungi. Sample salinity was not a factor in the ability of microorganisms to survive at the different pH levels. See Table 5 for more details.

Hydrocarbon-utilizing microorganisms can lower the water bottom pH by producing organic acids (Hill, 2000). SRB can raise the water bottom pH by removing the organic acids that are produced by the hydrocarbon-utilizing microorganisms.

5.5.6 Microbial contamination in fuel and the fuel distribution system

Development of microbial contamination in a fuel storage tank

The development of microbial growth in a fuel storage tank, especially with extended fuel storage, may occur in the following sequence. This growth sequence may be different in aircraft (subsonic and supersonic) and marine vessel fuel storage tanks due to their unique operating conditions.

1. Microorganisms are introduced into the fuel storage tank from the air, in water or in the fuel added to the tank. There could possibly be up to 10^5 microbial cells per cm^3 of air (Bento and Gaylarde, 1998). The storage

Table 5 The effect of pH on microorganism survival in sludge samples collected from ship storage tanks employing seawater compensated systems

pH of sludge water ^a	Predominant microorganisms recovered	Microorganisms present in lower numbers	Microorganisms inhibited
< 4	Yeasts & molds numerous	Bacteria rare	Bacteria, including SRB
4-8	Yeasts & variety of molds	Fewer bacteria than at pH > 8, SRB sometimes present	—
> 8	Bacteria, SRB ^b common	Variety of molds present (but dormant ^c)	Yeast & molds

(Table prepared from the results of a study by Neihof and May, 1983.)

^aSludge was centrifuged and the pH of the aqueous supernatant was measured with a combination glass-reference electrode.

^bSRB = sulfate reducing bacteria.

^cdormant but viable, could be recovered on culture media.

tank contains the bulk fuel, a small amount of accumulated water bottom and oxygen in the tank headspace or dissolved in the fuel (Figure 2).

2. Aerobic, hydrocarbon-utilizing (oxidizing) microorganisms start to grow at the fuel/water bottom interface. See Table 6 for a partial list of the wide variety of microorganisms that are capable of using hydrocarbons for growth. Bacteria and yeast normally colonize the system before the filamentous fungi (Bento and Gaylarde, 2001). Certain microorganisms, in contact with hydrocarbons, can alter their cell membrane structure by forming surface fimbriae and protrusions. It has been theorized that these changes enable the cell to better attach to the hydrocarbon droplets (Ratledge, 1988). Also, hydrocarbon utilizing microorganism cell wall surfaces are covered with a polysaccharide fatty acid complex that promotes the adhesion of these microorganisms to the fuel/water bottom interface (Smith, 1991). At this interface, the microorganisms have access to an energy source from the fuel hydrocarbons and utilize nutrients, trace elements and water from the water phase. The fuel/water bottom interface is a limited environment for microbial growth. In an effort to increase their habitat, microorganisms adapt a variety of different strategies.

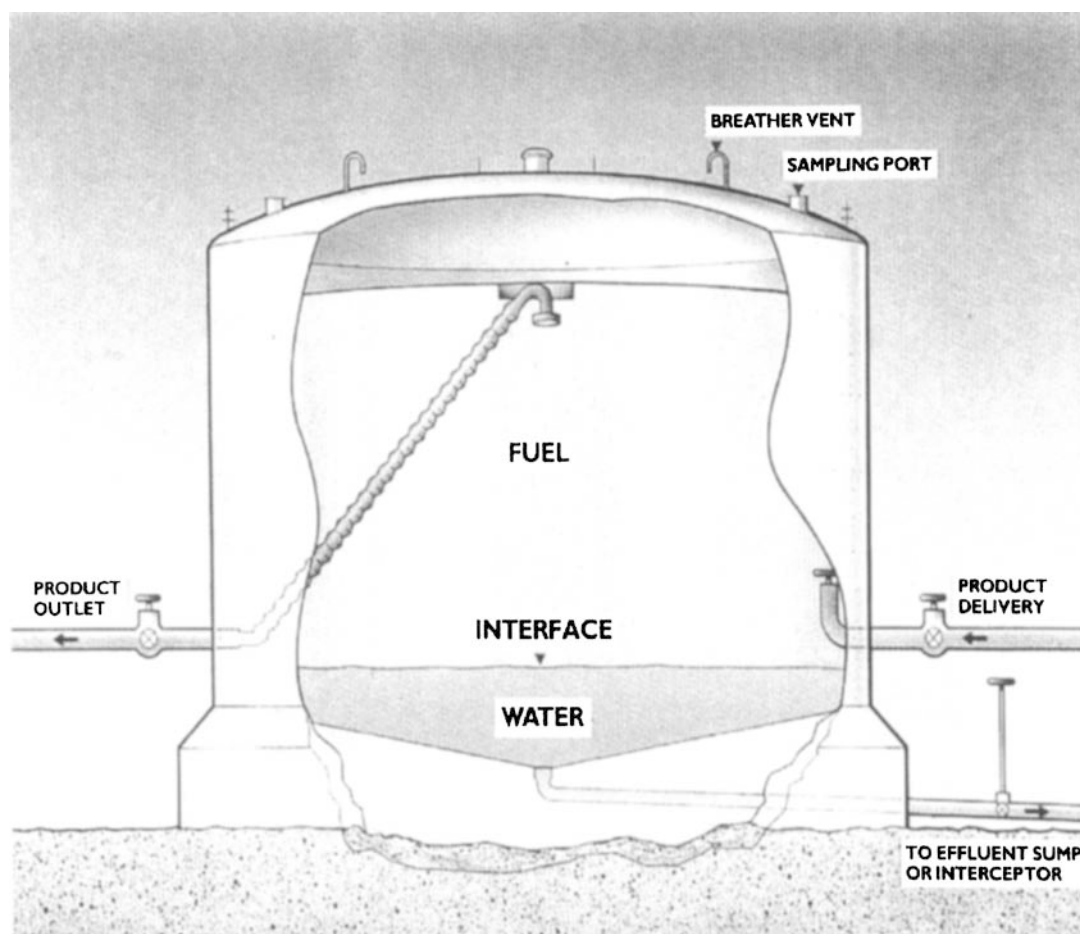


Figure 2 Development of microbial contamination in a fuel storage tank starts with the accumulation of free water under the fuel. (Graphic courtesy of the Rohm and Haas Company, Philadelphia, Pennsylvania, U.S., Copyright the Rohm and Haas Company.)

Table 6. A partial list of hydrocarbon-utilizing microorganisms

Molds	Yeast	Bacteria
<i>Acremonium</i> sp.	<i>Candida fumata</i>	<i>Acinetobacter calcoaceticus</i>
<i>Aspergillus fumigatus</i>	<i>Candida guilliermondii</i>	<i>Acinetobacter cerificans</i>
<i>Aspergillus</i> spp.	<i>Candida lipolytica</i>	<i>Alcaligenes</i> spp.
<i>Cephalosporium roseum</i>	<i>Candida rugosa</i>	<i>Arthrobacter paraffineus</i>
<i>Cladosporium cladosporoides</i>	<i>Candida tropicalis</i>	<i>Arthrobacter simplex</i>
<i>Fusarium</i> sp.	<i>Rhodotorula</i> sp.	<i>Bacillus</i> sp.
<i>Fusarium moniliforme</i>	<i>Torulopsis colliculosa</i>	<i>Corynebacterium glutamicum</i>
<i>Fusarium oxysporum</i>	<i>Yarrowia tropicalis</i> ^b	<i>Desulfovibrio desulfuricans</i> ^c
<i>Hormoconis resinae</i> ^a		<i>Nocardia petroleophilia</i>
<i>Mucor</i> sp.		<i>Mycobacterium smegmatis</i>
<i>Paecilomyces</i> sp.		<i>Pseudomonas aeruginosa</i>
<i>Paecilomyces variotii</i>		<i>Pseudomonas fluorescens</i>
<i>Penicillium</i> spp.		<i>Pseudomonas oleovorans</i>
<i>Penicillium corylophilum</i>		<i>Pseudomonas putida</i>
<i>Penicillium cyclopium</i>		<i>Pseudomonas</i> sp.
<i>Phialophora</i> sp.		<i>Rhodococcus</i> spp.
<i>Rhinochadiella</i> sp.		
<i>Trichoderma viride</i>		
<i>Trichosporon</i> sp.		

(Information from Hill and Thomas, 1975; Neihof and May, 1983; Bartha and Atlas, 1987; Smith, 1991; Gaylarde et al., 1999.)

^aFormerly *Cladosporium resinae*

^bFormerly *Candida tropicalis*

^ca sulfate reducing, anaerobic bacteria

- Microorganisms can increase the area of fuel/water interface.
Certain bacteria and yeast produce extracellular biosurfactants that emulsify water into the fuel above the interface. The bacteria and yeast can invade the fuel phase in the water droplets (Figure 3) dispersed in the oil emulsion (Smith, 1991).
- Microorganisms can transport water up into the fuel to permit growth in the fuel.
a. Some molds (*Hormoconis resinae* and *Phialophora* sp.) develop “feeding hyphae”, hyphae which have a thin film of water surrounding them. Droplets of emulsified oil can be found in this water envelop. The feeding hyphae extend up into the fuel phase (Smith, 1991).

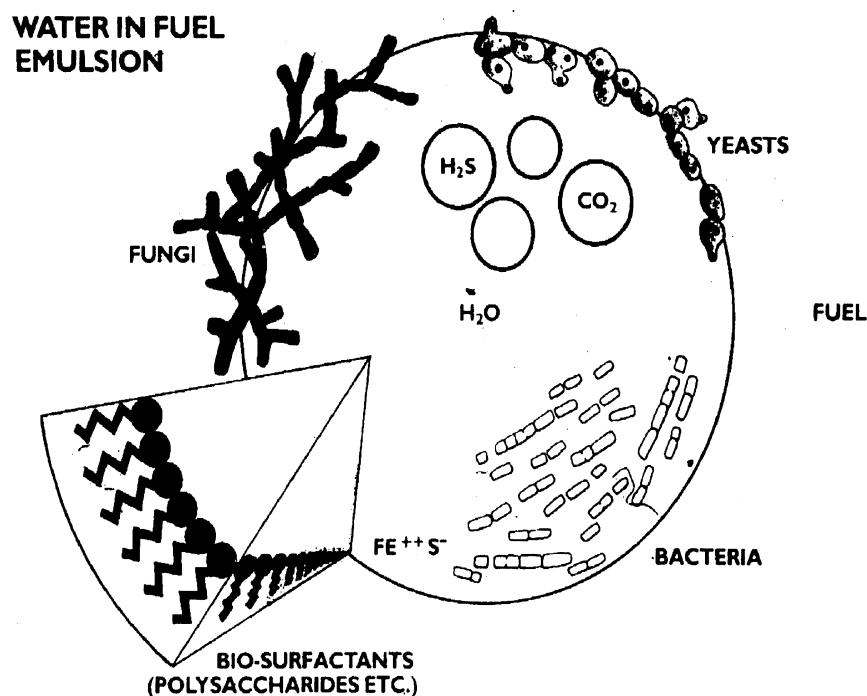


Figure 3 Microorganisms can invade the fuel phase in a water droplet, emulsified in the fuel by biosurfactants produced by microorganisms. (Graphic courtesy of the Rohm and Haas Company, Philadelphia, Pennsylvania, U.S., Copyright the Rohm and Haas Company.)

- b. Some bacteria can produce surfactant molecules that polymerize and form layers of thin sheets at the interface of the fuel and water. These layers accumulate to produce a slime layer into the fuel. Bacteria can attach to the sheets to move into the fuel and the polymer absorbs water from the interface for their needs (Smith, 1991). The polymer can attach to tank surfaces and thus secure water droplets to the tank wall that remain even after water bottom draining.
3. The hydrocarbon-utilizing microorganisms produce partially oxidized compounds (organic acids, carboxylic acids and alcohol) that can be used for growth by other aerobic (non-hydrocarbon oxidizing) microorganisms introduced into the system. This is a key function of the microbial consortia. *Aerobacter aerogenes*, *Enterobacter cloacae*, *Micrococcus* sp., *Moraxella* sp., *Ochrobactrum anthropii* and *Serratia marcescens* are some of the bacteria that have been isolated from fuel storage systems that are incapable of using hydrocarbons as their sole source of carbon for growth (Bushnell and Haas, 1941; Gaylarde *et al.*, 1999).
4. The microbial growth (biofilm) at the interface traps dust, grit, metal oxides, extracellular polymeric substances (EPS) produced by microorganisms, fuel degradation and oxidation products (i.e., gums), and metal swarf.
5. Eventually, a heavy growth of microorganisms produces a heavy layer of biomass at the fuel/water bottom interface. At the interface, a white layer called a "milk layer" is formed by the accumulation of fatty acids from the degradation of alkanes.
6. Sections of the floating biomass, containing microbial cells, dirt, dust and metal oxides, fall to the bottom of the tank (Figure 4). At the tank bottom, the biomass traps organic and inorganic debris to create sludge. Sludge can provide a protected, microenvironment for microorganisms, where growth conditions (oxygen, pH, nutrients, etc.) may be different from conditions in the rest of the tank (Neihof and May, 1983). Anaerobic conditions can develop under the sludge. Anaerobic conditions are also produced in some parts of the water bottom where the aerobic microorganisms, already growing in the storage tank water bottom, deplete the oxygen.
7. The anaerobic conditions in the tank water bottom can lead to the growth of anaerobic microorganisms such as SRB, i.e., *Desulfovibrio* spp., *Desulfotomaculum* spp. and *Desulfobulbus* spp. The SRB utilize the partially oxidized organic acids produced by the aerobic microorganisms for growth (Hill, 2000). SRB produce toxic levels of hydrogen sulfide that inhibit growth of fungi (Neihof and May, 1983) and cause blackening of waters due to iron sulfide complexes.
8. Under certain conditions (i.e., poor housekeeping), ciliate protozoa (*Bodo* sp.), flagellates and amoeba protozoa (bacteria predators) and even metazoan organisms such as nematodes (protozoa predators) are present in

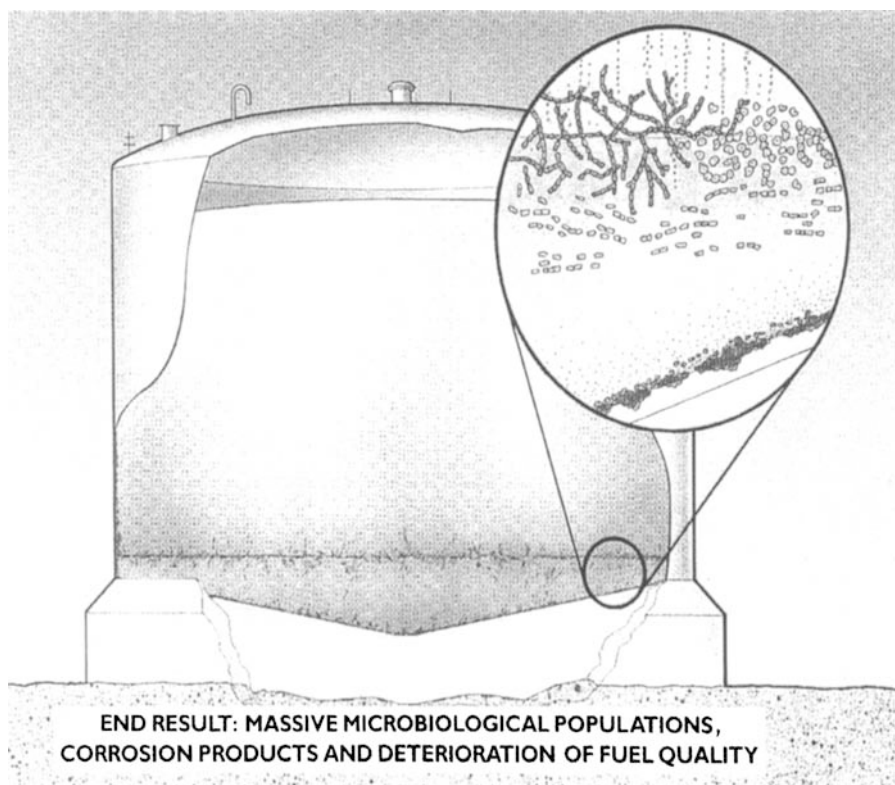


Figure 4 Microorganisms produce a heavy layer of biomass at the fuel/water bottom interface and in the fuel storage tank bottom. (Graphic courtesy of the Rohm and Haas Company, Philadelphia, Pennsylvania, U.S., Copyright the Rohm and Haas Company.)

the water bottom. The presence of these organisms indicates that the fuel storage tank has been contaminated for a long period of time. Algae are not present in the water bottom due to the lack of light to stimulate growth.

Hormoconis resiniae

One of the earliest and most common microorganisms isolated from contaminated fuel storage tanks is the mold *Hormoconis resiniae* (formerly *Cladosporium resiniae*). Because of the prevalence of this mold in the fuel distribution system, *H. resiniae* deserves special mention. *H. resiniae* metabolizes fuel hydrocarbons with chain lengths of 8 to 20 carbons (Genner and Hill, 1981). It has been isolated from jet, light distillate and marine gas turbine engine fuel systems and tolerates the repeated freeze/thaw cycles of subsonic aircraft fuel tanks very well (Hill and Thomas, 1975). *H. resiniae* has the ability to sporulate in fuel and the spores can survive for several years without water (Gardner, 1971). In fact, *H. resiniae* can grow in fuel at 25°C with only 80 ppm of free water (Hill and Thomas, 1975). The optimum temperature for growth is 25°C to 30°C (Park, 1975). *H. resiniae* grows best in water bottoms where the pH ranges from 3.5 to 6.0 (Hill and Thomas, 1975). The unattached hyphal growth at the fuel/water interface forms a thick fungal mat (Figure 5). The mycelium attach to the fuel tank wall forming a black, glutinous film. This growth is difficult to remove even by physical means. On agar media, *H. resiniae* colonies can range from pinkish-brown to gray-brown to olive green (Miller and King, 1975).

Changes in microbial populations

In the last twenty years, there was been a shift in the prevalent population of microorganisms found in contaminated fuel systems from the mold *Hormoconis resiniae* to bacteria such as *Pseudomonas* species, *Alcaligenes* species

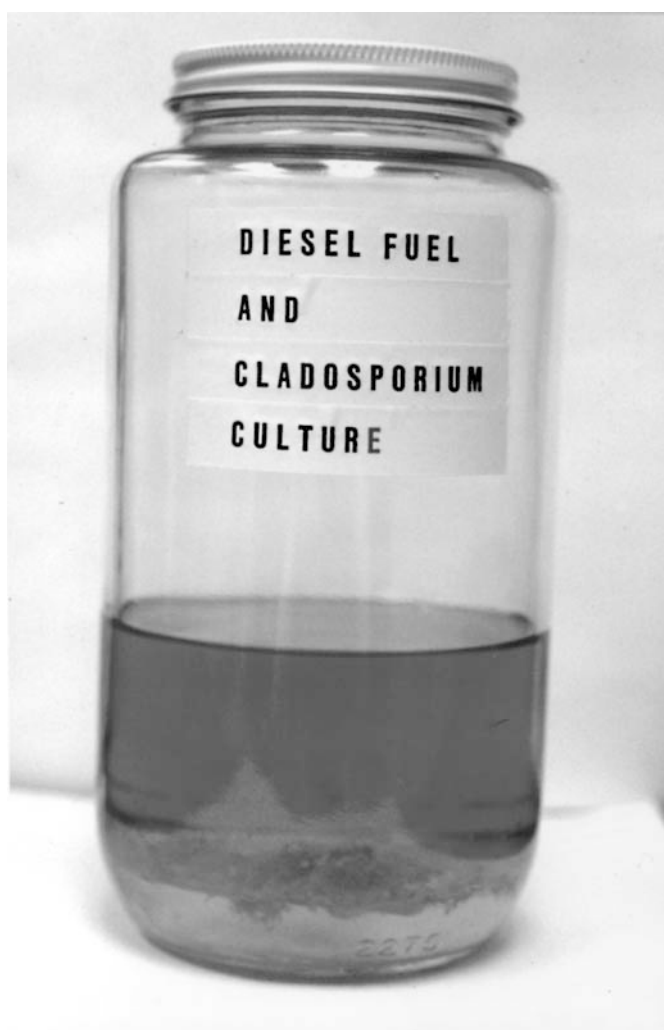


Figure 5 *Hormoconis resiniae* (formerly *Cladosporium resiniae*), growing at the interface between the darker diesel fuel phase at the top and the lighter water phase on the bottom, produces a thick grey to black microbial biomass.

and sulfate reducing bacteria (Smith, 1991). This may be due to longer storage times for fuel, increased awareness of microbial contamination and/or changes in the fuel chemical composition. These changes in fuel composition include the removal of lead, the switch from higher to lower aromatic compounds and the inclusion of oxygenated additives to fuel. It has been reported that *Aspergillus fumigatus* has been isolated as frequently as *H. resinae* from contaminated aviation fuel systems worldwide (Genner and Hill, 1981).

The total microbial population and the frequency with which microbial types are encountered in the contaminated fuel storage tank fluctuate as the conditions in the tank change. Microorganisms exist in a consortium; interacting, cooperating and competing with one another for growth nutrients and growing space. This competition and/or codependence will affect the growth rate and the types of microorganisms present. Quantitative changes can occur with changes in temperature, water concentration and inorganic nutrient concentrations and with fresh fuel addition. Qualitative changes can occur depending on the type of fuel stored, the oxygen conditions present in the water bottom, water pH and the age of the stored fuel/water bottom system.

Sampling procedures can also affect the type and frequency of microbial contaminants recovered from a contaminated fuel storage tank. The collection of samples, using a fuel sampler, provides data only on the planktonic population present in the tank water bottom and the fuel/water bottom interface where floating biofilm could be sampled. This sampling provides no information on the possible large sessile population attached to the walls of the tank. In many cases the actual level of contamination present is underestimated or misrepresented.

An extensive evaluation of microorganisms recovered from fouled diesel fuel systems conducted by Levy and Hegarty (1991), involving 400 samples from more than 100 distribution and service station tanks, demonstrated that viable aerobic, mesophilic bacteria were present in 75% of the water phase samples tested. The bacterial counts in these water samples were 10^5 to 10^6 colony-forming units per milliliter of sample (CFU/ml). Bacteria were recovered from 60% of the fuel phase samples, with maximum counts of 10^4 to 10^5 CFU/ml. Viable yeasts were present, at 10^3 CFU/ml, in 30 to 45% of the water phase samples and in 30% of the fuel phase samples. Viable molds were detected in 20 to 25% of both the water and fuel samples, with counts up to 10^3 to 10^4 CFU/ml. SRB were detected only in the water phase in approximately 10% of the samples.

Significant levels of microbial contamination

There are no accepted universal standards to define what constitutes significant microbial contamination in fuel or fuel storage tank water bottoms. Different advocacy groups involved with the fuel market, such as the International Air Transport Association (IATA), the Institute of Petroleum (IP) in the U.K. and the ASTM in the U.S., have suggested guidelines. The fuel transporters, distributors and end users all have their own requirements, relevant to their specific situations.

One fuel purchaser considers counts of $< 10^2$ CFU/L bacteria and $< 10^3$ CFU/L fungi as acceptable in their diesel fuel for a storage time of two to three years (Institute of Petroleum, 1996). The acceptable levels for jet fuel are one log lower for the same storage conditions. When over three years storage is required, the acceptable levels are lower still.

Both the Institute of Petroleum and the ASTM acknowledge the lack of generally accepted criteria for what would be considered significant microbial contamination. The Institute of Petroleum indicates that microbial counts in the water bottom of 10^9 – 10^{11} CFU/L bacteria, 10^7 – 10^{10} CFU/L yeasts, 10^6 CFU/L molds and 10^5 CFU/L SRB, either alone or in combination, are considered to represent microbial contamination (Institute of Petroleum, 1996). The ASTM suggests that counts of 10^5 CFU/ml can be, in many cases, accepted in the water bottom but any microorganisms detected in the fuel should suggest the need for corrective measures (ASTM, 2001). The ASTM also cautions that microbial counts should be routinely monitored and if problems with fuel chemistry, performance or physical appearance are also noted then corrective action should be considered.

In a study of aviation turbine fuel storage by Ferrari et al. (1998), successive fungal counts of greater than 50 CFU/L in the fuel were considered significant and meant that preventative measures should be initiated. Swift (1988) observed that 10^6 to 10^7 CFU/ml bacteria and 10^4 to 10^5 CFU/ml fungi could be detected in a heavily contaminated water bottom. Microbial mats or sediments could contain up to 10^6 to 10^8 CFU/gram wet weight of bacteria and fungi.

Microbial contamination in subsonic aircraft and their handling systems

Jet aviation fuel for subsonic aircraft is usually stored in the aircraft wings. Water condenses from humid air on contact with the cold wing structure. In flight, fuel temperature conditions are very cold. Temperatures can reach -40°C at 9,000 meters (30,000 feet) (Park, 1975) and the water freezes thus prohibiting growth of microorganisms. There is little effect on fungal viability at these cold temperatures; *H. resinae* and *Aspergillus niger* spores can remain viable from -32°C to 80°C (Thomas and Hill, 1977). When the aircraft is on the ground and the water thaws, temperatures in the tank can reach 25° to 40°C (depending on location) (Genner and Hill, 1981), temperatures at which microorganisms can readily grow. This is especially the case in underutilized aircraft, military and private, that remains on the ground with residual fuel stored in the tanks (Smith, 1991) and in aircraft in use in tropical climates.

Microbial contamination in supersonic aircraft and their handling systems

No serious reports of microbial contamination have been reported as yet in supersonic aircraft fuel systems (Genner and Hill, 1981) due primarily to strict fuel specifications, strict fuel handling procedures, good housekeeping and good tank design which allows for water elimination. However, the potential does exist for microbial contamination to occur. The environment in the fuel tank on supersonic aircraft is very different from that on subsonic jet aircraft. Fuel is also stored in wing tanks; however, the fuel is heated by friction during supersonic flight with air passing over the wing surface (Genner and Hill, 1981). Also, some of the supersonic aircraft systems discharge heat into the fuel. The fuel temperatures in flight can range from 20°C to as high as 100°C in some of the outer tanks (Genner and Hill, 1981), with an average high temperature of 50°C (Hill and Thomas, 1975). Normally, temperatures do not drop low enough for fuel to freeze as they do during subsonic flight. To maintain proper flight trim, fuel is switched back and forth from tank to tank. Under these conditions, the fuel temperature can be approximately 40°C, a temperature at which some microorganisms can grow, in some parts of the tank. An albino strain of *Aspergillus fumigatus* has been shown to successfully tolerate these diverse temperature conditions (Hill and Thomas, 1975). *H. resinae*, a major contaminant in subsonic aircraft, does not grow well at 40°C.

Marine vessels with water compensated fuel storage systems

Microbial contamination problems surfaced with the use of gas turbine engines in marine vessels (Genner and Hill, 1981). Seawater is pumped into an empty storage tank. As fuel is depleted, the seawater is pumped into the fuel tank to displace the spent fuel. There is a mixing of seawater with fuel in this tank until eventually the tank is completely seawater (Neihof and May, 1983). This replacement serves two purposes: (1) to maintain the ship's stability by providing ballast and (2) to eliminate vapor space in the tanks. This introduces microorganisms into a fuel tank with a high water to fuel ratio and temperatures ranging from 4°C to 25°C (Genner and Hill, 1981). *Hormoconis resinae* and SRB are the major contaminants of this system. However, as mentioned previously, *H. resinae* is usually found together with *Candida* sp. (which conditions the water pH to enable *H. resinae* to grow). *H. resinae*, not found in seawater, is introduced to the system in contaminated fuel (Neihof and May, 1983). SRB, imported with the seawater, thrive in anaerobic conditions under the fuel at the tank bottom and reduce the seawater sulfates to sulfides for energy (Neihof and May, 1983). *Paecilomyces*, *Fusarium*, *Aspergillus*, *Penicillium*, *Rhodotorula*, and *Pseudomonas* have also been recovered from marine storage tanks (Neihof and May, 1983).

The ballast water, with possible residual concentrations of biocide, is discharged into the sea with refueling. Therefore, it is important that any potential biocide selected for use in this application be environmentally acceptable and meets all appropriate regulations.

5.5.7 Consequences of microbial contamination

The consequences of microbial contamination have been classified into five categories: (1) problems caused by the physical presence of microbial growth, (2) problems caused by microbial metabolism, (3) problems caused by microbial metabolites, (4) problems caused by contact with sludge and (5) microbially induced corrosion. These problems are pertinent for aircraft, marine vessels, road vehicles and ground fuel storage.

Under most conditions, the contact time between the fuel and microbial contamination in the water bottom phase is too short and too limited for chemical changes to occur in the fuel (Herbert et al., 1987). There is a high volume of fuel to water and the fuel is deficient in the essential nutrients needed for microbial growth. The microorganisms for the most part are limited to the relatively small area of the water bottom and the fuel/water interface. However; with prolonged fuel storage, especially in a poorly maintained storage tank, the fuel chemistry could be affected.

(1) Problems caused by physical growth of microorganisms. Filamentous fungi and exopolymer producing bacteria cause the formation of thick fungal mats or bacterial biomass and slime that can cause machinery malfunctions due to physical blocking of pipelines, filters and small apertures. This biomass can also block drainage points in the tank, preventing the removal of water. On aircraft, fuel gauge malfunction can be caused by microbial slime accumulation around the fuel tank probe. Most of the problems encountered on aircraft are caused by fungal growth. In small boats, automobiles and trucks, microbial slimes/biomass can cause engine failures due to fuel starvation and fuel pump and filter clogging. Coalescers, installed to remove water, contain cellulose fibers that are subject to microbial attack. Development of microbial slime on the coalescers can impede water removal and eventually block the fuel flow.

(2) Problems caused by microbial metabolism. Fuel additives may be broken down by microorganisms to obtain nutrients needed for growth. This could degrade performance additives and decrease fuel efficiency and/or stability.

(3) Problems caused by microbial metabolites. Microorganisms can produce corrosive byproducts such as organic acids (acetic acid), hydrogen sulfide, sulfuric acid and ammonia. These corrosive byproducts can damage metal walls and fittings. These byproducts also degrade complex organic materials such as hoses and tank seals as well as accelerate sediment formation (Neihof and May, 1983). Sulfate reducing bacteria can cause a black precipitant (FeS) and sulfide souring of fuel. In an enclosed system, microorganisms can cause excessive gas production. The buildup of hydrogen sulfide in a closed storage tank can present health and safety issues (Hill, 2000). Eventually hydrogen sulfide goes into equilibrium between the water and the air in the tank headspace. However, a few ppm of hydrogen sulfide in the water can correlate to a lethal hydrogen sulfide concentration (> 700 ppm) in the air. Hydrogen sulfide can be severely irritating at 100-300 ppm and cause dizziness at 300 ppm (Hill, 2000). Other microorganisms may oxidize the sulfide to sulfuric acid. Certain aerobic bacteria are capable of producing biosurfactants that cause fuel/water emulsification. These biosurfactants reduce superficial tension and form micelles that accumulate at the fuel/water interface. They can cause water to be emulsified into the fuel, which can affect fuel burn or ignition properties (Herbert et al., 1987) and potentially cause coalescer malfunction. Some bacteria can cause moisture haze in fuel due to the formation of water droplets less than $1\ \mu\text{m}$ in size in the fuel phase (Smith, 1991). Bacteria can produce insoluble polymeric material in the fuel.

(4) Problems caused by contact with sludge. When floating biomass falls to the bottom of the tank, it traps organic and inorganic debris to form sludge. Components of the sludge can oxidize fuel to cause fuel degradation, corrosion and deterioration of polymeric materials in the fuel storage and transfer system. Sludge at the fuel/water interface can provide a barrier to decrease or prevent fuel soluble biocides partitioning from the fuel into the water bottom. Biocides can be absorbed into the sludge particulate surfaces and/or be inactivated by sludge components (Andrykovitch and Neihof, 1987).

(5) Microbially induced corrosion. Corrosion is an electrochemical process in which a charge difference develops in the electrical potential of adjacent areas of the storage tank metal surface. The water bottom in contact with the metal surface of the storage tank creates many micro areas acting as anodes and cathodes. Changes in the type and concentration of ions, pH values and oxygen levels can modify the electrochemical behavior of these electrical cells. Electrons will flow from the anode (area of lower potential) to the cathode where they are consumed by different reactions (water and oxygen, water and hydrogen ion, hydrogen and sulfate, etc.) depending on the nature of the environment. At the anode, pitting corrosion is initiated by the loss of metal ions into solution ($\text{Fe}^0 \rightarrow \text{Fe}^{+2} + 2\text{e}^-$). Microbially induced corrosion is usually caused by the activity of a community of microorganisms. This activity includes the degrading of organic substrates to produce potentially corrosive materials and the production of corrosive byproducts such as H_2S . The following processes can cause corrosion.

- Microbial layers (sludge) on metal surfaces can cause metal pitting or corrosion due to differing charge potentials between the covered and uncovered areas. Biopolymers in the biofilm trap ions creating a concentration of ions in the covered area. This will shift the potential of the metal surfaces to create localized corrosion cells. The area of lower concentration will be attacked.
- Fungi, such as *H. resinae*, and bacterial fermentations produce organic acids. These acids drop the water bottom pH and cause corrosion of metal tanks. This pH drop also causes weight loss of aluminum. Organic acids can penetrate protective tank coatings and remove them from the metal surfaces. This can indirectly aid in corrosion by producing bare patches of metal exposed to the corrosive environment, that become a small anode (uncoated area) connected to a large cathode (coated area). This causes rapid metal loss.
- SRB (mainly *Desulfovibrio* and the more oxygen tolerant *Desulfotomaculum*) reduce sulfates in the water bottom to produce H_2S , HS^- and S^{2-} , which are aggressive to steel and yellow metals. Sulfide can be reoxidized to form sulfuric acid (Hill, 2000).
- Some microorganisms can use the phosphate and nitrate components in corrosion inhibitors for growth. In doing so, they effectively remove the corrosion protection and indirectly aid in the corrosion process.
- Aerobic microorganisms in slimes or cracks use up the available oxygen, creating an oxygen deficient area. This area is anodic compared to an adjacent area with few microorganisms and oxygen present. This discrepancy leads to an oxygen gradient with electron flow from the oxygen poor area to the oxygen rich area. The electron loss causes anodic pitting corrosion to develop in the area with the low oxygen concentration. (Hill, 2000).
- Many microorganisms, including SRB, produce the enzyme hydrogenase that can depolarize metal surfaces by removing hydrogen directly. The surface becomes more porous and with hydrogen ingress it becomes hydrogen embrittled (Hill, 2000).

5.5.8 Signs of microbial contamination

Microbial contamination should be considered if there are complaints of machine or engine power loss or complete failure, signs of corrosion or slime formation in the fuel storage tank or on filters, clogged filters, fuel odor complaints or a reduction in fuel performance. One or more changes in the ASTM fuel quality specifications (i.e.

pour or cloud point, sediment, water content) could possibly indicate the presence of microorganisms. Other things could cause these changes but microbial contamination should also be considered (Passman, 1994). A visual check of the storage tank or lines can reveal the presence of a fungal mat or slime on the inside tank surface, slime around the tank access lids, and damage to tank coatings or pitting corrosion on the tank metal surfaces.

A visual inspection of contaminated fuel taken from a storage tank and allowed to settle in a clear storage bottle would show no visible change in the upper and middle portion of the fuel. There may be a moisture haze layer in fuel above the fuel/water interface. Fragments of hyphae may be present between the moisture haze layer and the fuel/water interface. Immediately above the fuel/water interface there may be a layer of emulsified oil droplets. At the interface, one would see a thick layer of microbial slime. The water bottom may be turbid and black with a strong hydrogen sulfide odor (Smith, 1991).

5.5.9 Prevention and clean up of microbial contamination

Routine maintenance

The first and most important approach in preventing microbial contamination in fuel is good housekeeping. This cannot be stressed enough. Problems detected early on are easier and less costly to deal with than when the system becomes heavily fouled by microorganisms. Cleanliness, scheduled routine maintenance (including water and organic debris removal, monitoring for the presence of microorganisms and sediment) can help decrease the chances of heavy microbial contamination problems. Inspection procedures should be documented, including the places, timing and method of sampling to be done. Maintenance records and appropriate corrective actions should be included. Despite all precautions, in reality it is virtually impossible to have a fuel storage system that is completely water free.

Water removal

Water is the most prevalent contaminant of the fuel storage system. Preventing and/or removing water is the key to having a fuel system without microbial contamination and the major component in good housekeeping. Remove the water and the microorganisms cannot grow and subsequently cause damage.

The basic sediment (BS) and water (W) content in fuel tanks should be tested routinely (ASTM Method D1796). The water content in light distillate fuel, present in the form of dispersed water droplets in hydrocarbon, can be measured by a water separator index modified (WSIM) test. The WSIM test indicates the potential for water formation in fuel emulsions. Corrective action should be taken if the BS and W levels are elevated, the WSIM levels are elevated or there are complaints from customers of filter, lines or nozzle plugging. ASTM also describes two methods ("Clear and Bright Rating") to determine free water and particulate contamination in distillate and middle distillate fuels (ASTM, 2000b; ASTM, 2000c).

Fuel storage tanks should be designed to facilitate water removal. Tanks should preferably be slightly sloped or have a cone down construction to allow for water accumulation at the lowest point of the tank. There should be a drainage line at this low point, seated flush with the tank bottom, and a sump pump for water removal. If the tank has a flat bottom, there should be multiple drains spaced strategically around the tank bottom. Fixed roof tanks are desirable. Before fuel is transferred to a new storage facility, it should be filtered (5 μ m filter) to remove particulates and run through a water separator (coalescer) to remove any free water. Centrifuging capability on incoming fuel lines is also advisable. Piping should also have drains at the lowest point.

Other means used to reduce water in the fuel storage tank include a nitrogen gas blanket over the fuel to reduce moisture, desiccants placed in the tank vents to remove moisture in the air, and periodic recirculation of the fuel through coalescers to remove water and foreign material. Filters should be checked regularly and cleaned as needed. Also, a check for microbial contamination in the fuel should be made before addition to any new tank.

An above ground tank is easier to service than an underground tank, as it is difficult to remove water from an underground tank. However, temperatures are more consistent in an underground tank and tank location and fuel volumes to be stored may make their use more preferable. At service stations, the gasoline and diesel tanks are located underground and are generally pitched at an angle. If the sounding and fill tube is located at the high end of the tank, the presence of a water bottom, microbial contamination and accumulated sediment may erroneously go undetected (Passman, 1994). Records accumulated from over 1,000 retail tanks cleaned demonstrated this situation in 50% of the tanks (Chesneau et al., 1995). Also, the sump pump drain tube may be raised up above the tank floor or not located at the lowest point of the tank. Under these circumstances, it would be impossible to drain out all of the water from the underground tank (Passman, 1994).

Industry controls

The aviation industry requires very high standards for jet fuel maintenance. Jet fuel is filtered every time it is moved along the distribution chain – even up into the aircraft itself. Water is removed from the wing tanks routinely. New aircraft integral wing tank design deters water accumulation by reducing horizontal surfaces and corners (Genner and Hill, 1981). Some storage tanks for aviation fuel have a floating suction tube that draws fuel off the top of the tank rather than from the bottom where the microbial contamination, water and particulate matter are concentrated.

Fuel storage tanks at nuclear power plants (regulated by the Nuclear Regulatory Commission) must be cleaned every ten years. If excess debris is found, these tanks are cleaned more often.

Use of biocides

A biocide is a chemical compound or mixture of chemical compounds that can kill microorganisms at recommended use levels. The use of biocides for both preventative (maintenance dosing) or curative (shock dosing) treatment of fuel and fuel storage systems has proven to be very successful, cost effective and safe. Biocides are formulated to protect a product or a system from incoming microbial contamination. Their efficacy is severely challenged in very heavily contaminated systems where microorganisms are being constantly reintroduced to the system. If a system contains a heavy biomass or sludge, this material should be removed first before biocide treatment. Biocides may not be able to penetrate deeply into the biomass to reach all the viable microorganisms and some components of the biomass may trap or deactivate the biocide. Biocide treatment will be discussed in greater detail in the next section of this chapter.

Tank cleaning

In heavily contaminated systems, biocide treatment alone may not be sufficient to address the problem. Melton et al. (1988) found that a combination of mechanical centrifugation/filtration of distillate fuel followed by biocide treatment provided the best results for long-term fuel storage protection from microbial contamination. It may be possible to remove particulate matter, including microbial contamination, by recirculating the fuel through 0.5 micron filters (Chesneau, 1991). The contaminated fuel system should be kept static for an appropriate period of time (approximately a week) to allow the contaminants to settle out in the tank bottom before filtration and separation is initiated (Rogers and Kaplan, 1968).

In situations where the microbial contamination is so heavy that filters would be ineffective due to clogging, it may become necessary to remove the fuel from the tank and perform a physical cleanup (pressure washing and scrubbing) to remove biomass/sludge (Figure 6) and debris from the tank (Chesneau, 1991). After the tank has been cleaned and sanitized, clean fuel can be added and new biocide can be dosed for protection. Disinfectant and sanitizing agents selected for use must be compatible with the construction materials of the storage tank. However, physical tank cleaning is a very labor intensive and expensive process and there are a lot of safety issues involved. It also requires shutting down the tank for whatever time it takes to clean it up and return it to service, which could be a financial loss. To reach the whole tank for cleaning, scaffolding may need to be erected. The tank must be degassed first to protect people entering the tank. The volatility of gasoline fuel creates additional safety issues when gasoline tanks require cleaning (Chesneau et al., 1995). Filtration systems used to clean these tanks must be explosion proof, grounded and have an emergency shutoff. Emergency fire protection should be readily available. There are some robotic devices to clean the inside of the storage tank, but they have not proved to be effective. Safety regulations for this type of cleanup vary depending on the locale.

Alternate cleaning processes

The process of “fuel polishing” is used to clean contaminated fuel on marine vessels (Johnson, 1998). The contaminated fuel (including water and sludge) is pumped from the tank, filtered and then returned clean to the tank. Service companies can provide this service at larger ports or provide the necessary equipment for use on the boat.

Magnetic devices are currently being marketed to remove microbial contamination from marine and diesel fuel. Fuel contaminated with microorganisms is circulated through a unit containing a strong magnet or a series of magnets. These devices claim to work by disrupting the cell membrane leading to the impairment of microbial metabolic and reproductive functions and ultimately to cell death as the microorganisms pass through the magnetic field (Johnson, 1998). Some of the manufacturers of these devices advertise that the unit breaks up microorganisms into tiny particles that easily pass through the filters to be burnt with the fuel in the engine. There is presently only limited acceptance for this concept and technology.



Figure 6 Cleaning sludge, including microbial biomass, from the inside of a contaminated fuel storage tank. (Picture printed with the permission of Howard Chesneau, Fuel Quality Services, Flowery Branch, Georgia, U.S.)

5.5.10 Biocides for the protection of fuel storage systems

Biocides used to protect fuel and the fuel storage system fall into the categories of fuel-soluble, which exhibit varying degrees of solubility in both the fuel and the water phases, or water-soluble only biocides. The method of biocide addition will depend on the type of biocide selected. Commercial biocide manufacturers recommend a treatment range to cover both preventative (maintenance) dosing and curative (shock) dosing. With little or no microbial contamination present in the fuel storage tank, the biocide is used at the preventative levels. When heavy microbial contamination exists in the tank, a higher concentration of the biocide is needed for eradication. It is very important that biocides are dosed according to their manufacturer's directions. Using lower than recommended biocide levels (sub-lethal doses) can provide nutrient sources for bacteria and actually increase microbial contamination. Also sub-lethal biocide dosing can potentially lead to a microbial resistance problem. The biocide should be adequately mixed into the system for optimum activity. After biocide dosing of a heavily contaminated fuel storage tank, it may be necessary to remove the dead microorganisms and biomass that fall to the tank bottom. There have been many studies evaluating the efficacy of biocides in protecting fuel/water systems (i.e., Rogers and Kaplan, 1968; Andrykovitsh and Neihof, 1987; Dorris and Pitcher, 1988). From a regulatory perspective, biocides must be approved by various regulatory agencies including the U.S. EPA, the European Biocidal Products Directive (98/8/EC) and the Pest Management Regulatory Agency of Health Canada. See Chapter 4.

A biocide for fuel treatment should have the following characteristics (Rogers and Kaplan, 1968; Dorris and Pitcher, 1988; Gaylarde et al., 1999):

- Toxic to microorganisms at low levels,
- Broad spectrum of antimicrobial activity at use levels,
- Good rate of kill (a significant reduction of microorganisms within 24 hours is desirable),
- Stable over a range of applicable temperatures (18°C to 57°C),
- Cost effective,
- Cause no adverse effects on engine performance,
- Non-corrosive at use levels to fuel tank components (metals, fuel tank coatings, etc.),
- Low ash content (ash can damage the fuel injection system and cause combustion chamber deposits),
- Engine manufacturers' approvals,
- Regulatory approvals for use,
- Acceptable human toxicity at use levels,
- Environmentally acceptable – biodegradable,
- Easy and safe to handle.

Table 7 Common biocide chemistries used to treat both the fuel and the fuel water bottoms

Active Ingredient
5-Chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one (CMIT/MIT)[II, 15.3.]
3,5-Dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thiono (DMTT) [II, 3.3.25.]
1-(2-hydroxyethyl-2-alkyl(C-18)-2-imidazoline
N,N'-methylene-bis-(5-methyl-oxazolidine) [II, 3.3.9.]
Methylene bis(thiocyanate) (MBT) [II, 20.9.1.]
4-(2-nitrobutyl)morpholines + 4,4'-(2-ethyl-2-nitrotrimethylene) dimorpholine [II, 3.2.3.]
2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane [II, 9.11.] + 2,2'-(1-methyltrimethylenedioxy)-bis-(4-methyl-1,3,2-dioxaborinane)
Polyolefin + Boric acid [II, 8.2.1.]
2-(Thiocyanomethylthio)benzothiazole [II, 15.11.] + Methylene bis(thiocyanate)
1,3,5-Triethylhexahydro-s-triazine [II, 3.3.19.]

Fuel-soluble biocides

Fuel-soluble biocides can be used to treat the contents of the whole storage tank, both fuel and water. As mentioned previously, these biocides are soluble in both phases at varying concentrations. These biocides can be added to a fuel storage tank as follows: (1) through a port at the top of the tank, (2) premixed with fuel outside the tank and pumped back into the tank, (3) injected into a flowing stream of incoming fuel, or (4) added to fuel inside the tank to which more fuel will be added. If biocide solubility in fuel is low, the premix method of addition is not recommended. Methods of addition that insure uniform distribution of the biocide in the fuel and water bottom are obviously preferred. It is not recommended to add the biocide to an empty fuel storage tank. The tank should be at least 10% full prior to biocide addition.

The advantages with fuel-soluble biocides are that they reach most areas of the tank and even after partitioning into the water bottom some amount remains in the fuel to provide protection when the fuel is moved down through the distribution system (Dorris and Pitcher, 1988; Williams *et al.*, 1992). Fuel-soluble biocides may take a little longer to kill the microorganisms because they must first partition from the fuel into the water bottom where the microorganisms are concentrated. Because the biocide is added to the fuel portion, these biocides must have all the necessary government and engine and airframe manufacturer's approvals. See Table 7 and **Part II*** for common chemistries of fuel and water bottom treatment biocides.

Additional characteristics desired for a fuel-soluble biocide are (Rogers and Kaplan, 1968; Dorris and Pitcher, 1988; Gaylarde *et al.*, 1999):

- Adequate fuel and water solubility,
- Ability to migrate to the water phase (where the microorganisms grow),
- Good chemical stability in fuel,
- No formation of soaps or emulsions with fuels,
- No negative effect on the fuel or fuel specifications,
- Good compatibility with fuel additives (anti-icing agents, corrosion inhibitors, anti-oxidants, etc.).

Water-soluble biocides

Water treatment biocides are added directly to the water bottom. The cost to treat is less than for the fuel-soluble biocides because the volume to be treated is much smaller. Because these biocides are added to the area which microbial growth takes place, they can start to kill immediately. No additional approvals are needed because water-soluble biocides do not enter the fuel or effect fuel properties (Dorris and Pitcher, 1988). It is often difficult to add a biocide just to the water bottom. However, if the biocide partition coefficient is low and the specific gravity is higher than the fuel, the biocide can be added at the top of the tank and it will go quickly to the water bottom (Dorris and Pitcher, 1988). On the negative side, water-soluble biocides cannot provide continual protection for the fuel while in storage or as it is moved down through the fuel storage distribution chain to the end-user. See Table 8 for common water-soluble biocide chemistries.

Water-soluble biocides should have these additional characteristics (Rogers and Kaplan, 1968; Gaylarde *et al.*, 1999):

- Good water solubility,
- Good chemical stability in water.

*see Part Two – Microbicide Data

Table 8 Common biocide chemistries used to treat the fuel water bottom

Active Ingredient
2-bromo-2-nitropropane-1,3-diol [II, 17.14.]
2,2-Dibromo-3-nitrilopropionamide [II, 17.5.]
4,4'-(2-Ethyl-2-nitrotrimethylene)dimorpholine + 4-(2-Nitrobutyl)morpholine [II, 3.2.3.]
Glutaraldehyde [II, 2.5.] + Oxydiethylenebis(alkyl dimethyl ammonium chloride [II, 18.1.]
Disodium ethylenebis(dithiocarbamate) [II, 11.12.1.] + Sodium dimethyldithiocarbamate [II, 11.11.1.]
Potassium dimethyldithiocarbamate [II, 11.11.2.]
1, 3, 5-Triethylhexahydro-s-triazine [II, 3.3.19.]

Biocide partitioning

At equilibrium, a successful fuel-soluble biocide should concentrate at effective levels in both the water and the fuel phases. Each time the fuel is transferred through the fuel distribution system, to a new storage tank with an existing water bottom, some biocide active ingredient will partition from the fuel into the water phase and remain there. This active ingredient loss will continue through each transfer of fuel. Therefore, when the fuel is moved to a new storage tank it will contain less biocide active ingredient than initially added. It is desirable to select a fuel-soluble biocide that can partition into the water bottom to provide microbial kill and still be present at effective levels in the fuel to protect the fuel throughout the distribution chain to the end-user (Figure 7).

The degree of partitioning is dependant upon the solubility of the biocide in each of the phases and is effected by the type of fuel, extent of mixing, contact time and the fuel to water ratio (Williams et al., 1992). The partition coefficient (K) of a substance is a measure of the relative solubility of a substance between two immiscible liquid phases. It can describe the affinity of the biocide for one phase over the other. For a fuel/water system:

$$K = \frac{\text{concentration in the fuel phase}}{\text{concentration in the aqueous phase}}$$

The log of the partition coefficient is often referred to as log P.

A biocide with a high partition coefficient favors the fuel phase over the water phase. Under these circumstances, higher biocide concentrations would be required to treat a system with a high fuel to water ratio of >1,000:1, to insure that adequate active ingredient levels reach the water phase to effectively kill the microorganisms (Dorris and Pitcher, 1988). With a low partition coefficient, the biocide would favor the water phase over the fuel phase. This biocide would be effective at low doses in treating a system with a high fuel to water ratio, because so much of the biocide partitions into the water phase (Dorris and Pitcher, 1988).

Aviation fuel biocides

Biocides are acceptable as an aviation fuel additive under ASTM D1655 (Jet A) with the provision that they have approval from the purchaser and airframe and engine manufacturers (Hill and Hill, 2000). The choice of a biocide for aviation fuel is limited to three specific approved chemistries:

- glycol ethers (EGME, DIEGME – primarily anti-icing agents with bacteriastatic properties)
- isothiazolone (3:1 mixture of methylchloroisothiazolone and methylisothiazolone) [II, 15.3.]
- organoborinane (mixture of two dioxaborinanes) [II, 9.11.].

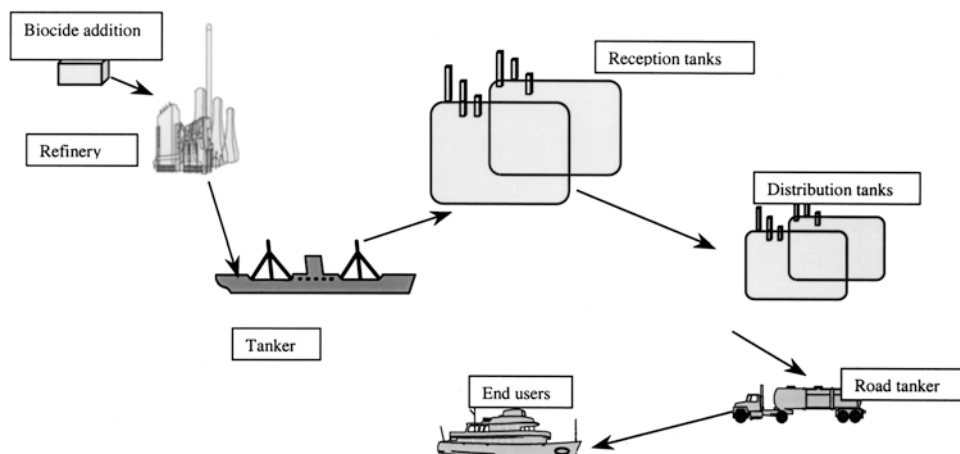


Figure 7 Adequate biocide protection is needed from the point of biocide addition at the refinery, through a typical fuel distribution chain, to the end-user.

5.5.11 Tests to determine microbial contamination in fuel systems

There can be problems assessing microbial contamination in fuel. Because fuel is a non-aqueous media, many types of assays to determine microbial contamination cannot be used directly, such as enzyme testing. Also, the actual number of microbial contaminants may be low (10^3 to 10^6 CFU per liter of fuel) which could complicate determining the level of microbial contamination. A large proportion of particles causing filter blockage may be non-microbial.

Fuel sampling

Every effort should be made to obtain appropriate samples for analysis. Microbial contamination in the fuel storage tank will not be homogeneous throughout the tank. Access to the tank is limited and this makes obtaining a representative sample a problem. Microorganisms can be found in many locations throughout the tank. Crane and Sanders (1967) reported that some microorganisms were able to move from the water bottom in a static tank to approximately 0.4 meters (14") above the fuel/water bottom interface after eight days. Microorganisms may be in biofilms on the tank surfaces and these microbes would not be detected in either a fuel or a water bottom sample (Passman, 1994). Therefore, test samples should be taken from multiple sites in the storage tank. Fuel from the top, middle and sides of the tank as well as at the fuel/water bottom interface should be sampled for microbial contamination. Water samples from the water bottom and the water bottom/fuel interface, as well as near or under any biomass in the water bottom, should also be evaluated for microorganisms. If there is sludge or biomass on the filters, then the presence of microorganisms should also be determined on a dry solids basis.

The Institute of Petroleum (1996) recommends that sampling be done routinely every one to six months or more often (at least twice over a three to five day period) if there is a problem.

An adequate amount of fuel, from 0.5 to 1 liter, should be evaluated for microbial contamination. It is important that the fuel sample reach the laboratory that is evaluating the fuel for microbial contamination within a maximum of 48 hours. Some microorganisms present may not survive for longer periods of time in the fuel phase. Only molds may be recovered from a fuel sample; fungal spores remain viable for many months, yeast for a few days and bacteria only for a few hours (Hill, 1975). Water bottom samples should also arrive at the laboratory as soon as possible. The loss of fuel contact could negatively affect some of the microorganisms that depend on a hydrocarbon source for growth. Also, fresher samples will yield more accurate qualitative and quantitative results. If the temperature increases, free water may dissolve in the fuel portion of the sample and viable microorganisms may be lost. To prevent this, samples should be kept refrigerated and processed as soon as possible.

Containers selected to transport fuel or the fuel/water bottom to a laboratory should be impervious to the fuel. If possible, sterile glass or plastic (i.e., high density polyethylene or polypropylene) containers are suggested. The Institute of Petroleum (1996) recommends using silicone rubber stoppers or plastic screw caps with inserts to cap these bottles. All samples should be properly labeled with the company name, the location of the tank, the location within the tank where the sample was taken, the type of sample collected (either fuel, water bottom or fuel/water bottom interface) and the date and time the sample was collected.

A bottom-sampling device, such as the Thief Sampler, can be used to obtain samples from the bottom of the fuel tank. This device has a clear thermoplastic tube with a bottom foot valve, which is operated from the top by a knob connected to the foot valve by a stainless steel control rod that runs the length of the device. Graduations on the tube exterior enable the operator to record where microbial contamination is found and the fuel tank level (Melton et al., 1988).

Agar medium plating for total viable plate counts

It is generally not necessary to determine the genus and species of the microbial contaminants isolated to determine if the fuel tank needs treatment or what treatment to provide. The approximate number of total viable microorganisms present in the system will allow you to make this decision. This can be determined by an agar plate count using selective media.

Agar plating of the fuel phase to recover microbial growth can be accomplished in two main ways: fuel filtration and fuel emulsification, prior to agar media plating.

(1) Fuel filtration. Fuel contamination can be collected on a hydrocarbon impervious 0.22 (for bacteria) or 0.45 (for fungal growth) micron filter (i.e. Millipore membrane type HA). Filter a set aliquot of fuel through the filter unit. Wash the filter with a detergent to break up any particulate matter and remove residual fuel from the filter. This can be done by using a 0.1% (v/v) sterile detergent solution and washing the filter with 10 washes of 10 ml each (Herbert et al., 1987). Follow this with three washes of 10 ml sterile saline (0.85% NaCl, w/v) or sterile buffer to remove the detergent. Place the filter, face up, on an agar media surface in a petri plate. Incubate the agar plate at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 48 hours and check for the presence of bacteria. Reincubate the plate for seven days to two weeks and check for the presence of fungal contamination.

(2) Fuel emulsification. Emulsify 1 ml of fuel in 9 ml of Ringer's solution with Tween 80. Prepare serial dilutions of the emulsion in sterile phosphate buffer and pour or streak plate the dilutions. If the fuel is viscous, 0.1 ml of fuel can be added to 0.2 ml of the emulsifying agent. Then add 9.7 ml sterile deionized water to make a 1:100 dilution. Plate this solution directly or prepare dilutions for plating on or with agar media. This method has a lower level of detection compared to the filtration method.

The fuel water bottom can be plated directly onto the surface of solid agar media or pour plated with molten (cooled to 50°C) liquid agar. If the microbial counts are suspected to be high, the water bottom should be diluted to 10^{-3} to 10^{-6} in an appropriate medium such as sterile phosphate buffer prior to plating. Sediment (0.01 to 0.1 g wet weight) can be dissolved in 100 ml of a suitable dilution broth prior to agar plating (Swift, 1988).

The Institute of Petroleum published the following test method, Standard IP 385/95, for fuel boiling below 390°C:

Procedure A: for enumeration of CFU/liter of < 25,000 and SRB,

Procedure B: for enumeration of CFU/liter of > 25,000.

A set volume of fuel is filtered through an appropriate sterile filtration unit. The filter can be evaluated for microbial contamination by directly placing it on agar media or suspending it in sterile liquid media to extract microorganisms and then plating the liquid media onto agar media (Institute of Petroleum, 1996).

The Institute of Petroleum also has a method to determine fungi in fuel, IP PM BY/95, Determination of Fungal Fragment Content of Fuels Boiling Below 390°C. This is also a filtration method where a known volume of fuel is filtered through a 0.45 or 0.8 µm filter with a grid of 3 mm squares. The number of fungal fragments per liter is calculated from the number of fungal fragments on the grid and the volume of fuel filtered (Institute of Petroleum, 1996).

Some media used to recover total viable microbial populations (bacteria, molds and yeasts) include Nutrient Agar, Plate Count Agar, Typticase Soy Broth Agar and Tryptone-Glucose Extract Agar. Fungi can be grown on Malt Extract Agar with Penicillin G (60 mg/L) and Rose Bengal (5 mg/L) (Ferrari, 1998), Mycosil Agar, Potato Dextrose Agar without supplements, Potato Dextrose Agar supplemented with 0.5% yeast extract, Sabourauds Dextrose Agar or YM (yeast, mold) Agar. Different additives can be added to the molten agar media on preparation to eliminate certain microorganisms that may have a negative impact on fungal recovery. To prevent bacterial growth, 0.001% w/v chlortetracycline, cyclohexamide or chloramphenicol can be added to the media. Potassium tellurite (0.01%) can be added to agar to inhibit the growth of *Candida* sp. to allow for better recovery of *H. resinae* (Rossmore et al., 1988). A problem associated with the agar plating method to recover microorganisms from a fuel system is that some microorganisms just do not grow on solid agar media.

Dip slide microbial growth detectors

This is an easy, semi-quantitative method to determine the total microbial count in aqueous solutions. Samples can easily be collected in the field by on-site staff. The Dip Slide detectors are plastic paddles coated with a different agar media on each side. Usually one side is a media for total count of microorganisms and the other side is a media selective for fungi. Some companies have added triphenyl tetrazolium chloride (TTC) to the media as an indicator dye, which is reduced from its original colorless state to an insoluble red formazan by most microorganisms. These microorganisms will appear to be red on the media, making it easier to count the colonies. The paddle is dipped into the test solution (water bottom), removed and allowed to drain to remove excess test solution. Paddles can also be used to check tank surfaces for microorganisms by pressing the agar against the surface. The inoculated paddle is incubated at 25°C or 30°C for 48 hours to seven days to recover bacteria and fungi respectively. The quantity of microbial growth on the dip slides is compared with a chart to determine the degree of microbial contamination present. The level of detection of microorganisms is typically 10^2 to 10^3 CFU/ml in water, depending on the system. There are many companies that manufacture these dip slides – i.e. Bug Check® BF from Avalon International Corp. (U.S.), Hycheck™ contact slides from Difco Laboratories (U.S.), Microtest® P manufactured by France Organo Chimique (France), Milliopore™ Samplers from the Millipore Corporation (U.S.) and Sani Chek from Biosan Laboratories, Inc. (U.S.).

Fuel samples can be tested with these dip slides by first emulsifying the fuel and then sampling the emulsion with the dip slide. With the AFNOR Test NFM 07-070 1993, fuel emulsified in water (1:10) is tested for growth with a dipstick. This test detects only bacterial growth > 10^5 bacteria/L (Institute of Petroleum, 1996).

Test kits for the detection of microorganisms

There are also a number of microbial detection test kits available for use. Some of these test kits are described below.

Air BP MicroMonitor² was developed by ECHA Microbiology in the U.K. to determine the quantity of microorganisms present in a non-aqueous (fuel or lubricants) or aqueous sample. A measured amount of fuel

or water bottom is added to the glass test bottle containing a thixotropic microbial culture gel and a growth indicator. The test bottle is shaken and the gel liquefies. Test samples are incubated for six days. Microbial growth turns purple and can be easily counted or the amount of growth can be compared to a control chart for an estimation of contamination levels (ECHA Microbiology Ltd, 2002).

FuelSTAT[™] resinae was developed by Conidia Bioscience, Ltd. for the rapid (10 min) identification of *Hormoconis resinae* in aviation fuel. This is a semi-quantitative immunoassay test that will determine if the level of *H. resinae* contamination is below the level of significant contamination, per the IATA Microbial Contamination Task Force recommendations. This test has the sensitivity to determine the presence of 1,000 µg of *H. resinae* biomass in 1 liter of fuel or 1 ml of water. Test results of negative, low positive or high positive correlate to < 1000, 1,000 to 10,000 or > 10,000 µg of the fungus in either 1 liter of fuel or 1 ml of water respectively. The manufacturer of this test kit suggests that an aviation fuel system be monitored at least once a year. However, if the aircraft travels in warm, tropical areas it is advisable to monitor once a month. If the test indicates a low positive contamination, some action may be needed and monitoring should be done more often. With a high positive test result, immediate remedial action is required (Conidia Bioscience Ltd, 2002).

HUM-Bug Detector[®] Kit, manufactured by Hammonds Fuel Additives, is a qualitative test that indicates that hydrocarbon utilizing microorganisms are present. The test flask contains the fuel or water bottom sample to be tested, a hydrocarbon source, an aqueous phase with nutrients added and a color indicator. The test bottle is incubated at 25°C in the dark for 12 to 48 hours. A red or pink color indicates the presence of hydrocarbon utilizing microorganisms (Hammonds Companies Inc., 2002).

RapidChek[®] II SRB Test Kit is manufactured by Strategic Diagnostics, Inc. (U.S.). This test kit detects the presence of SRB in water bottom samples in less than twenty minutes. Antibodies are used to detect the enzyme adenosine-5'-phosphosulfate (APS) reductase, which is produced by all strains of SRB.

Sulfate reducing bacteria can be detected and counted using API RP38 Sulfate Reducer Media supplied by C & S Laboratories, Incorporated (U.S.). The sample to be evaluated is injected with a sterile syringe into a small glass bottle, with a sealed rubber septum, containing API RP38 SRB media and a small nail. The medium provides a reduced, anaerobic environment needed for SRB growth. The bottles of broth (a series of six are needed for microbial counts) are incubated for approximately seven days at 30° to 32°C. SRB will produce sulfide that will react with the iron nail in the bottle to produce a black color (iron-sulfide precipitate).

Other microbial test kits to determine microbial contamination in the fuel and water phases or to detect the presence of biocides are recommended by the Institute of Petroleum (Institute of Petroleum, 1996).

Microscopic observations

Fuel and water bottom samples should routinely be observed under the microscope to determine if microorganisms are present. Non-viable microorganisms can be detected with this observation. Even if these microorganisms are not viable on receipt of the sample, they may have produced harmful metabolites in the system. Also, not all microbial contamination can be cultured on standard agar media. Non-microbial debris (dirt, dust and rust particles) will also be present, making it hard to identify microorganisms. Bacteria, molds (mycelial fragments) and yeast as well as microscopic parasites can be found in the water bottom. Bacteria in the water bottom may be hard to identify under optical microscopy (reflected and transmitted light) unless they are motile. Non-motile bacteria and spores can be stained with a fluorescent stain and viewed with epifluorescent microscopy (Neihof and May, 1983). Bacterial and fungal spores may be found in fuel.

Scanning electron microscopy can identify microorganisms by shape and size. Fungi, such as *Hormoconis resinae*, will appear as interwoven, filamentous structures with branching approximately 2 µm in diameter (Westbrook *et al.*, 1988). Yeast will be oval-shaped, some with budding, 1-2 µm in diameter. Bacterial rods, such as *Pseudomonas* sp., will be 0.5-1 µm wide and 2-3 µm long. Some bacteria form long chains of cells or filaments.

Immunofluorescence

This is a rapid (detection within four hours) and sensitive method to identify microbial contaminants. However, you must have the appropriate equipment and trained personnel to run the test and interpret the test results. Immunofluorescence was evaluated by Gaylarde *et al.* (1998) as a method for identifying *Hormoconis resinae* in aviation kerosene fuel. There was little cross-reaction with other fuel fungi. The hyphae and spores of *Hormoconis resinae* were detected in a biofilm.

Catalase determination (Passman, 1994)

This is a rapid test (15 min) that detects microbial growth based on the principle that many microorganisms produce the enzyme catalase that converts hydrogen peroxide into oxygen and water. A test sample is added

to 30% (v/v) hydrogen peroxide in a stoppered reaction tube. After 15 minutes, the pressure in the headspace of the reaction tube, due to oxygen buildup, is read in an HMB Instrument (BioTech International, Houston TX.). Test results are compared with controls. This test method follows the patented procedure of Kraft et al. ("Apparatus for Testing the Contamination of Industrial Liquids", U.S. Patent 4281536, 1981). The pressure buildup is proportional to the microbial load. Significant microbial contamination is indicated by a pressure reading of > 1.0 psig (after subtracting out non-catalase derived pressure from a control). This method is useful; however, it is a relative measurement of microbial contamination, since not all bacteria and fungi produce the enzyme catalase.

Biocide efficacy testing

Many laboratory test methods have been proposed to evaluate and determine the efficacy of biocides in fuel storage systems. Because these methods do not have the ability to simulate fuel storage systems, particularly reproducing the fuel/water ratio conditions, it is recommended that laboratory test results be confirmed with a field trial. For most of the methods proposed, the inoculum consists of three hydrocarbon-utilizing microorganisms: one bacterium (*Pseudomonas aeruginosa*), one mold (*Hormoconis resinae*) and one yeast (*Yarrowia tropicalis*). The inoculum count is approximately 10^7 CFU/ml bacteria and 10^5 CFU/ml for each fungal species. In the "South Africa Standard Specification for Biocides" (SABS) and NATO tests, non-hydrocarbon utilizing microorganisms are also included. None of the official methods mention the addition of SRB.

These tests are set up with the test fuel over a simulated water bottom. The ratio of fuel to the water bottom, mentioned in the literature, ranges anywhere from 1:1 to 1,000:1. The industry standard for the simulated water bottom is Bushnell Haas Mineral Salts Enrichment Medium (Bushnell and Haas, 1941).

The Department of the Army has a Performance Specification for Stabilizer Additive for Diesel Fuel, US MIL Specs 53021A, which describes testing for the performance of fuel biocides. The fuel/water ratio used in this test is currently 1/100 but 1/1,000 is also recommended. This test consists of a single inoculation with hydrocarbon-utilizing microorganisms. Metal coupons are added to the water bottom to check the ability of the microorganisms to cause corrosion in the system.

ASTM: E1259-01 – "Standard Test Method for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C is a multiple challenge test method. The fuel to water ratio decreases over time from 1/400 to 1/200 to a final ratio of 1/100 after eight weeks of the evaluation.

SABS 1434-1987, the "South Africa Standard Specification for Biocides", was reaffirmed in 2000. This method covers efficacy testing of three types of organic biocides (differentiated by their physical state – liquid, paste or solid) that are soluble in fuels and soluble in or miscible with water. These biocides are intended for use in hydrocarbon liquid fuels.

Specification NATO Stanag 7063: "Methods of Detection and Treatment of Fuels Contaminated by Microorganisms" is a single challenge test designed to evaluate biocide efficacy in aviation fuel. The fuel to water ratio recommended is 1/1000 and the inoculum consists of both hydrocarbon-utilizing microorganisms and non-hydrocarbon utilizing microorganisms.

Two valuable resources of information on fuel biodeterioration are ASTM D6469-99 (ASTM, 2001) and U.K. Institute of Petroleum (Institute of Petroleum, 1996) guidelines.

5.5.12 Conclusions

Water contamination in the fuel storage tank is inevitable. This is a fact of life for fuel manufacturers, distributors and end users. With water comes the potential for microbial contamination. A wide range of microorganisms is readily able to contaminate the fuel storage tank, which provides all of their essential growth requirements. These microorganisms are able to survive in a wide array of environmental conditions that may be encountered. It has been demonstrated repeatedly, that microbial contamination can cause severe problems in the fuel distribution storage system. These problems are due primarily to biomass and sludge development, the production of biosurfactants and corrosive metabolites and hydrogen sulfide generation.

The best way to prevent or reduce the impact of microbial contamination is to initiate a program of good housekeeping with routine monitoring for microbial contamination, water removal, filtering for particulate matter and dosing with a biocide to provide protection for the fuel and the fuel storage tank system. It is also very important to educate the individuals who maintain these systems. The importance of proactive routine maintenance and the ramifications if microbial contamination does occur should be stressed. Procedures should be documented, including corrective actions to be taken if a problem arises. It is easier, less expensive and lower risk for the maintenance crew to prevent, rather than fight, a heavy microbial contamination problem in the fuel distribution storage system.

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5.6 Microbicides for coolants

W. SIEGERT

5.6.1 Introduction

When using water-mixed coolants, technical and hygiene problems, whose origins lie in microorganism invasion and growth are something that even today practically all users have to prevent.

There are many ways in which microorganism can invade the freshly prepared water-mixed coolants, e.g. through dirty water, waste, tramp oils, air, the work-piece, operating personnel, and inadequate industrial and production hygiene (Figure 1).

If one considers coolant formulations, it can be seen that all elements necessary for microorganism growth – carbon, nitrogen, sulphur, phosphorus and trace elements – are plentifully available as a result of the coolant components. Thus amines serve as a source of nitrogen, mineral oils and emulsifiers as a source of carbon, and the sulphate and sulphonate groups contained in anionic emulsifiers as well as the sulphate from the water serve as a source of sulphur. The plentifully available water and oxygen in the use-solutions permit aerobic metabolic processes, and in the case of standing coolant solutions or those with little movement they permit anaerobic metabolic processes. An average circulating temperature of approx. 40°C then creates optimum conditions for the growth and multiplication of microorganism.

The effects of uncontrolled microbial activity in water-mixed coolants can essentially be described as follows:

1. Effects on the coolant
 - breakdown of components
 - formation of breakdown products
 - reduction of the pH value
 - decreased protection against rust
 - increase in conductivity
 - formation of nitrosamines
2. Effects on the circulating system
 - separation of the oil
 - blocking of pipelines
 - occurrence of foam
 - difficulties in filtration
3. Effects on the environment
 - development of odour
 - danger to health
 - increase in complaints about skin irritation
4. Effects on other systems
 - interference with ultrafiltration when disposing of the coolants
 - interference with ultrafiltration in cleaning plants
 - interference with ultrafiltration in electro-coating plants

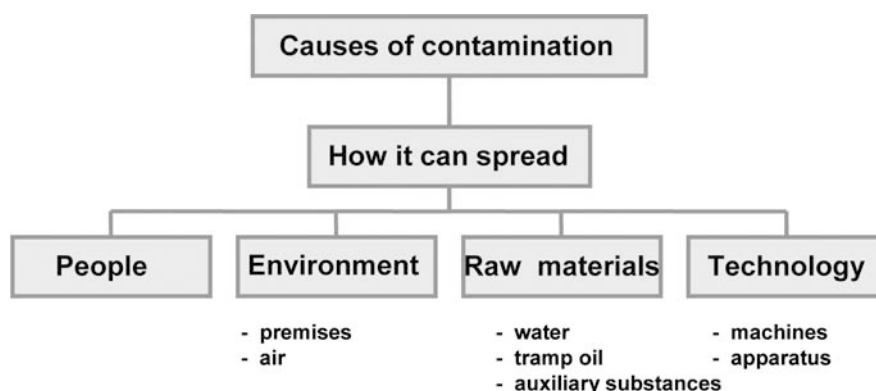


Figure 1

From this description it can be seen that control of the microbial growth in water-mixed coolants is necessary in order to considerably improve their standing times.

The microorganism counts of the order of 10^6 – 10^8 microorganism/ml in common coolants that are mentioned in some of the recent literature are not acceptable with regard to technical problems. With counts of between 10^2 and 10^4 microorganism/ml for bacteria, in most cases a plant can be operated without technical problems. With 10^2 – 10^4 cfu¹/ml of mould fungi, the situation can be quite different. The microorganism – particularly in the case of mould fungi – are often not evenly distributed in the coolant circulating system, but occur in the form of clusters or clumps, as a result of which spontaneous contamination can frequently be caused.

Also from aspects of human hygiene, especially for dermatological reasons, the number of general pathogenic bacteria should not be greater than 10^4 cfu/ml. Coolants should not contain any epidemic-causing pathogens.

5.6.2 Care of coolants

5.6.2.1 General

Care of coolants is understood to mean a number of measures that together should make possible the optimum use of coolants.

The first condition is the careful planning and construction of circulating plants. Depending on the work process, appropriately designed cleaning equipment must be included. Questions about emulsion-cooling or filings treatment and transport must be answered. Questions of demulsification and elimination of the used coolants must be resolved for ecological and economic reasons.

5.6.2.2 Preservation

5.6.2.2.1 General. The measures that must be taken for the control of uninhibited microbial growth in water-mixed coolants can in general be summarised under the heading “preservation”.

The aim of preservation is to protect a given product or medium against microbial material degradation for a sufficiently long period of time in the prevailing local conditions.

In principle, there are two possible ways:

- physical control
- chemical control

Physical treatment methods using e.g. brief heating, ultrasound or radiation are still at a trial stage to some extent. Also the use of bacteria filters in combination with oligodynamic copper ions or the use of ozone also fail in practice on account of costs being too high, inadequate effectiveness or a lack of technology. Also, a 90% reduction in the microorganism count achieved by centrifuging coolant solutions is not adequate in practice.

Chemical preservation using antimicrobially acting chemicals is today the most widely used control method.

Among the list of care measures, chemical preservation makes an important contribution towards ensuring the maximum technical performance of apparatus and plant within the circulating system by excluding or helping to reduce the malfunctions caused by microorganism.

5.6.2.2.2 System cleaning. The microbiological conditions in a coolant system (and this also applies for individual machines) can best be controlled and kept within reasonable limits over a long period if hygiene measures are applied when changing the use-solutions. Only through a sensible combination of cleaning and disinfection of the plant before refilling, and subsequent preservation, can the best standing times be achieved. A residual amount of just 20 l of highly contaminated emulsion with a microorganism count of 10^7 cfu/ml in a 20 m³ system means an initial count of 10^4 cfu/ml in the fresh emulsion (Figure 2). Particularly critical are the slime coatings formed by microorganism, in which in some cases counts of 10^9 cfu/g are found. They constitute a constant re-infection potential. Under such conditions, no long-term effect can be achieved, even with specific preservation measures, as penetration into the contaminated coatings is always inadequate. See chapter 6.1.

Compared with the method of separate cleaning and disinfection in two operations, the use of system cleaners for cleaning and disinfection in a single operation is a considerable advance. In addition to the application technology advantages, there is also the improved economy. During the system cleaning of a coolant carrying system, the circulating use-solution is reduced to the lowest possible level. Then a system cleaner in a concentration of 0.5–1.0% (in a heavily contaminated plant up to 3.0%) is added. In these products, special wetting agents ensure good wetting even of places that are difficult to reach. They loosen dirt, clumps of fungi and clusters of bacteria

¹ colony-forming units

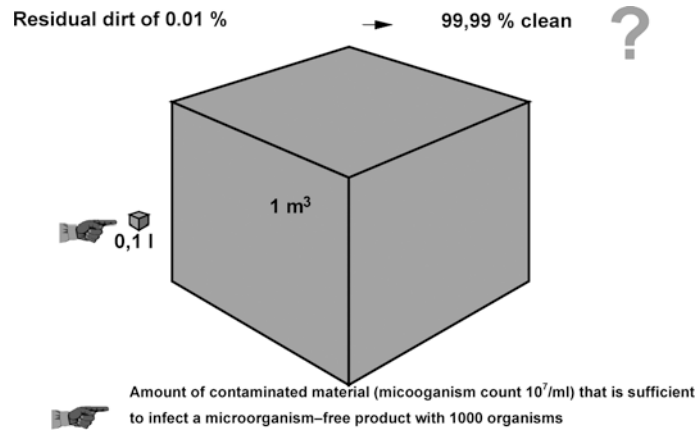


Figure 2

from the substrate. The incorporated emulsifiers carry the milky emulsion oil and loosened dirt and e.g. clumps of fungi that are present in the system. Over the course of approx. eight hours, microbicides contained in the system cleaner ensure a reduction in the microorganism count in the system such as is shown in Figure 3.

If the operating procedures involve coarse working, work can be continued during use of the system cleaner. In order to prevent blockages, monitoring of screens and overflow connections is advisable.

As can be seen from the course of the microorganism count, in the short term there is even an increase in the count, caused by the loosening and opening up of massive clusters of bacteria. But after only two hours a clear reduction in the microorganism count can be seen. The microbiological decontamination, i.e. the killing of the organisms in the system, is accompanied by a very good cleaning effect. As mentioned earlier, deposits are eliminated from the system, and thus new microorganism entering the system are deprived of at least some of the nutrient medium. After the end of the contact phase, which normally lasts a shift, the coolant solution containing the system cleaner is discharged as usual, demulsified and eliminated.

In this connection, it should be mentioned that the use of system cleaners can under certain circumstances make the demulsification of old emulsions more difficult. In the case of solutions that are still relatively fresh, i.e. containing an unused emulsifier proportion, system cleaners bring a markedly increased level of surfactant.

As a result of alkaline system cleaners, excessive alkalisation can also occur in the short term, as a result of which, e.g. for acidic demulsification, the amount of acid has to be increased.

During system cleaning, critical points that are difficult or impossible to reach with the emulsion – such as e.g. filter belts or perhaps one or other of the storage containers – must be cleaned separately. In particular, sumps must be drained.

After such system cleaning, good conditions for refilling the plant are created. The plant is largely free of microbes, and contains no residues.

However, the use of system cleaners cannot replace the later use of a preservative.

The cleaning process described here is applicable for the use of both alkaline and neutral system cleaners (e.g. when working with aluminium).

Course of the microorganism count during system cleaning

with Grotanol SR 1(schematic)

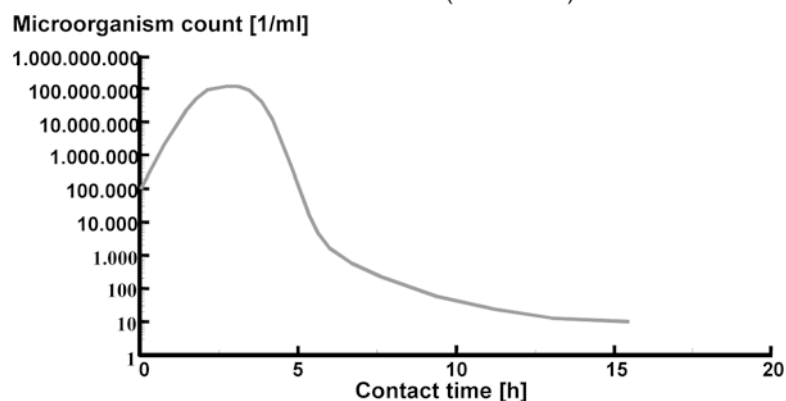


Figure 3

5.6.2.2.3. Preparation of the water diluted coolant. After the system has been cleaned, the new batch of metalworking fluid is prepared and the plant is refilled. Only faultless components should be used for the preparation of water-mixed coolants. In the case of coolant concentrates, it may be assumed that they are microbe-free. However, testing does no harm. Great attention should be paid to the water used for mixing. Surface water should never be used, as the danger of microbial contamination is greatest with this. Water from ion exchange plants is also frequently highly contaminated, especially if e.g. the exchange plant has not been used over a weekend. Major damage can often be avoided by determining the microorganism count before mixing. When preparing smallish amounts of metalworking fluid, which in most cases are not prepared by means of automatic mixing equipment, care must be taken to ensure that heavy contamination does not occur as a result of residual amounts of old solutions in the equipment.

5.6.2.2.4 Practical use of preservatives.

5.6.2.2.4.1 Fundamentals. Despite decontamination of the circulating system and new preparation of the emulsion, in practice the growth of microorganism after a short time cannot be ruled out. The following graph shows the multiplication of bacteria overnight (Figure 4).

In order to prevent the described effects on the coolant, the circulating system, production and the environment, in preservation practice chemical preservatives are used in concentrations of 0.05–0.2%, related to the end dilution of the coolant.

For the successful use of chemical preservatives in subsequent preservation, several essential points must be heeded.

In addition to the approximate chemical composition of the coolants, data such as discharge losses, evaporation amount, later addition of concentrate, use-concentration, pH value, nitrite compatibility and method of running the plant should be available.

In addition to microbistatic effectiveness, the selected preservative should also have a microbicidal effect. However, when evaluating the microbicidal effect, one should not apply the strict hygiene criteria of disinfection e.g. in hospitals, as otherwise for rapid disinfection in 30–60 min concentrations of 0.5–5% would have to be used, even for preservatives with a microbicidal effect. However, such high concentrations are not economically acceptable for coolants. In addition, the problem of skin tolerability plays an important role on account of the often intensive skin contact with water-mixed coolants. The presence of such high concentrations of preservative in the use-solutions would probably also lead to a significant change in the coolant properties. An accurate requirement here may therefore merely be appropriate microbicidal effectiveness in 1–6 hours.

With regard to the spectrum of effect of the preservative that is used – commonly also described as a “bactericide” – the result is the need for effectiveness against bacteria, yeasts and mould fungi.

In addition, the chemical compatibility of the preservative with the coolant must be ensured, and also, when various preservatives are used, the compatibility of these products with each other.

Depending on the method of operation of the plant and other specific features, the nature of dosing the preservative is established. There are three possibilities:

- addition via pre-preserved concentrates
- continuous addition to the water-mixed coolant
- non-continuous addition to the water-mixed coolant

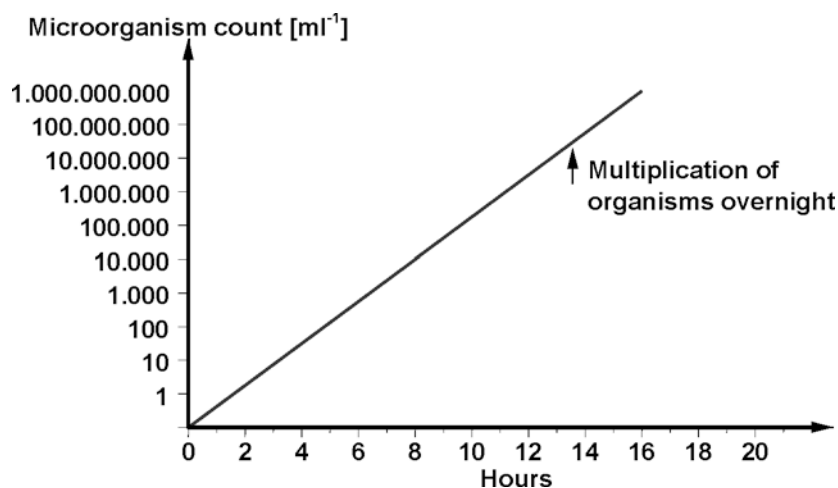


Figure 4

As a result of frequently varying use-concentrations of a coolant, depending e.g. on the work process, a standard preservative concentration cannot ensure microbiological effectiveness over the entire concentration range. In one case the concentration for adequate effectiveness is too low, in another case too high. The use of pre-preserved coolants is therefore possible only to a very limited extent, e.g. in individual machines.

The continuous addition of preservatives is only possible with central supply units, and is particularly advisable for those with a low discharge. The amount of preservative to be added is determined by means of routine tests at one- to two-week intervals. Satisfactory results are generally achieved in this way.

Preservatives are added at intervals mainly in plants with high discharge losses or in the case of individual machines in which continuous dosing would either not be practicable or would be too expensive. It is possible, at comparatively low cost, to ensure satisfactory operation of the plant by means of adding preservative at one- to two-week intervals.

In the case of dosing at intervals, the coolant is best added at the weekend during the last shift, provided that the coolant is kept in circulation over the weekend. In this way, any variations in pH or unpleasant smells for the people operating the machines can be kept as low as possible.

With regard to the practical aspect of adding preservative, it must be pointed out that the point of addition must be carefully selected. Care must be taken to ensure the most rapid and even distribution of the preservative possible. Points with great turbulence are the most suitable. Dead zones, or points near to filter belts or skimmers are not suitable. With filter belts or skimmers the risk of immediate discharge of the preservative is very great.

For an optimum effect of the preservative, constant presence of the active substance in the concentration that is technically necessary must be guaranteed. Both underdose and overdose must be avoided.

Underdose leads to:

- inadequate effect
- a false sense of security
- adaptation of the microorganism.

Overdose leads to:

- uneconomical work
- greater toxicity
- a greater burden on the environment.

The technically necessary use-concentration of the preservative can only be determined by means of biovalidation tests that are carried out in a laboratory, simulating practical conditions as far as possible, or, better still, in practical tests.

The correct concentration can also be jeopardised if e.g. the preservative has inadequate water solubility or too low a dissolution rate. Adsorptive properties can result in a great loss of the preservative, e.g. via the metal filings. A rapid loss of effect will also occur if resistance to alkali and temperature is inadequate. Water that is too hard, a high electrolyte content, and certain metal ions can in some circumstances contribute to a reduction of effect, as can too many organic impurities.

Keeping a monitoring record has proved very useful in practice for the constant monitoring of coolant circulating systems.

These records should include – in addition to the plant-specific – data such as type of coolant, machine, material pH value, corrosion values, oil level and amounts added – data on microbiological treatment measures.

These microbiological treatment measures include cleaning and disinfection activities for the circulation plants, and detection and avoidance of weak points in the system.

The information to be recorded should include microorganism count, rough differentiation of the flora into bacteria and fungi, preservative, preservative concentration, and amounts of preservative added.

In order to introduce preservation measures at the weekend, a sample of the circulating coolant can be taken e.g. on Mondays. By Wednesday or Thursday the appropriate microbiological information is available, so that the necessary steps for preservation can be instituted.

Depending on the coolant, the material being worked, the work process and e.g. the condition of the plant, it is advisable to carry out such a determination weekly or at three- to four-week intervals.

5.6.2.2.4.2 Development of resistance. At this point, the question of the “development of resistance” during the use of chemical preservatives will be briefly discussed. In the area of coolant preservation, if there is an apparent decline in effectiveness or inadequate effectiveness of the preservative, the concept “resistance” is too quickly, and wrongly, brought into the discussion.

As can be seen from the literature, technical preservatives attack the cells of the microorganism very non-specifically, with all structures or functions of the cells being attacked. Here lies the difference from antibiotics and chemotherapeutic agents, which attack cells specifically at quite specific sites.

It is impossible for the cells to protect themselves against a coolant-preservative's attack on many fronts. If, therefore, in practice microorganism survive after the use of preservatives, the reason can only be selection as a result of adaptation. Since the concentrations of preservatives that are necessary for inhibiting growth (microbistatic effect = reversible damage) and for killing microorganism (microbicidal effect = irreversible damage) are different, if the technically necessary use-concentration (determined in order to achieve irreversible damage) is not reached, individual microorganism that are no longer irreversibly damaged by the existing concentrations can survive. Since the majority of other microorganism are killed, these selected organisms find a space in which they have the ideal conditions for living and development.

The motto for the use of preservatives can therefore only be

“As little as possible but as much as is technically necessary”.

5.6.2.2.4.3 Toxicology of the preservatives. Questions concerning toxicology and other influences with regard to man and the environment are increasingly the centre of attention when chemical preservatives are evaluated, e.g. by hygiene and environmental consultants, and during their use.

Since the preservatives are chemical substances that are intended to attack, damage or even kill microorganisms, it is not difficult to imagine that they possibly also affect macroorganisms. In the case of such an active agent with polyvalent reaction possibilities, it is obvious that it does not differentiate between a microorganism cell and a macroorganism cell, and attack the one but spare the other.

It is clear from this that handling and using such substances can involve some risks. In particular, harm can occur as a result of contact with skin and mucous membranes. It is vital that the stipulated protective measures—especially when handling the preservative concentrates – are adhered to.

In order to be able to estimate the possible risks when working with chemical preservatives, before they are used in practice for the first time the toxicology should be evaluated on the basis of the expert reports that are available for the products.

After studying the relevant documentation, one will often find that commercial technical preservatives are not always highly toxic substances or preparations. The preservatives formulated with the appropriate know-how are highly effective products with very good toxicological properties. In addition, the so-called “relative toxicity” should be considered, i.e. the effect of the preservative on the total toxicity of the water-mixed coolant after dilution beyond the relevant use-concentrations, e.g. 1:1000.

5.6.2.2.4.4 Environmental-toxicological parameters. In a safety data sheet in accordance with 91/155CE, the most important data relevant to the environment, providing information on the effect of preservatives e.g. on the waste-water, should be shown. Knowledge of the COD value, which is most important in calculating possible damage, the BODS value, which permits conclusions regarding biodegradability, and on sewage sludge toxicity, which shows the effects on a biological sewage treatment plant, enable the user of preservatives to classify the products with regard to environmental-toxicological aspects.

The value for waste-water or sewage sludge toxicity indicates the maximum concentration of a preservative that has no harmful effect on the microorganism of the activated sludge flora, the biological stage of a treatment plant. From this, the factor by which the solutions of e.g. preservatives that are discharged into the waste-water must be diluted can be calculated. In general, these dilution factors are 1:50 to 1:200 in relation to the use-concentrations.

5.6.2.2.5 Easy-care coolants. Boric acid (II, 8.2.1.)* derivatives, e.g. boric acid ester, with different alkanolamines are used in these formulations. In practice, the content of these derivatives is between 20 and 40%. The boron content of such a product then varies between 0.5 and 2%.

Referring to such products as “biostable” formulations is, I believe, not appropriate, because otherwise it could be concluded that non-biodegradable raw materials are being used here.

It is well known that such boric acid esters have a growth-inhibiting effect on bacteria, but that the growth of yeasts and moulds is only inadequately suppressed. Thus these substances also fall under the definition of a chemical preservative.

A promotional claim such as “contains no preservatives” is therefore inappropriate for coolants formulated in this way.

Many users of such easy-care coolants are of the opinion that additional use of a chemical preservative is not necessary. We have investigated this question in extensive laboratory tests by subjecting such water-mixed coolants to a preservation challenge test (S&M Boko test; Figure 5).

Principle: In different test preparations, different concentrations of the preservative to be tested are added to the unpreserved samples. A constant microorganism load is achieved by periodic inoculation of the test preparations. During the inoculation period, just before inoculation, samples from the individual preparations

*see Part Two – Microbicide Data

S & M Boko Test

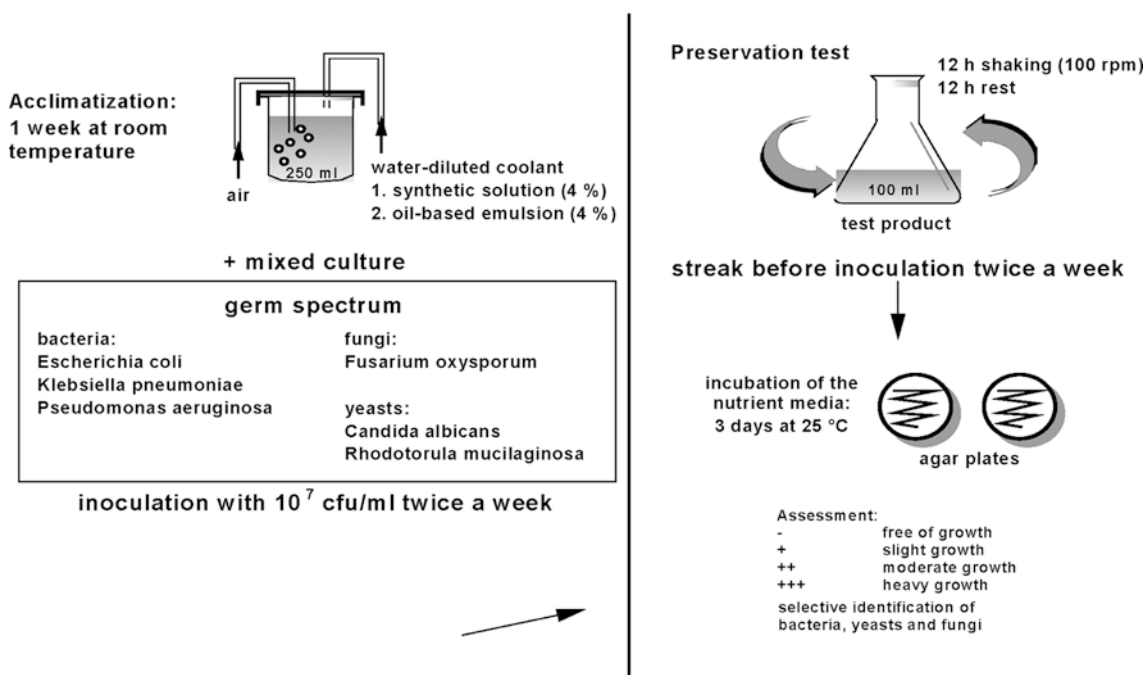


Figure 5

are streaked out. An evaluation is made on the basis of the microbial growth of the streaks. The longer the time to the first occurrence of microbial growth, the more effective is the preservative.

Method: In each case, 100 ml of the water-diluted coolant to be preserved is mixed with different concentrations of the preservative to be tested in separate preparations. An unpreserved sample serves as the growth control in each case.

Two days after incorporation of the preservative, the test preparations are infected for the first time with 1 ml of an inoculation solution. This inoculation solution is a mixture of gram-negative and Gram-positive bacteria and yeasts and moulds (isolated from various emulsions used in practice and cultured on nutrient media, then adapted to water-diluted coolants). The inoculation solution has a titre of at least 10^7 microorganism/ml.

The container containing the test preparations are left open, and are subsequently inoculated twice weekly and streaked out twice weekly onto agar plates, with the first streaking out being carried out just before the new inoculation. During the test, the test preparations are shaken for twelve hours per day and left undisturbed for twelve hours per day. The microbial growth on the streak preparations is evaluated after three days incubation at 22 °C. For the sake of caution, negative streak preparations are observed for a further two days and evaluated again. The preservative effect of the individual product concentrations is evaluated on the basis of the growth of the streaks, using the semi-quantitative method, with a score from – through + to +++.

The preservation challenge test is discontinued when +++ microorganism growth has been found consecutively on at least three streak preparations. After at most twelve inoculation cycles with negative results (–) the test is also discontinued.

The conditions of this test can be made harder by incorporating the preservative into the coolant concentrate, storing it for ten days at +40 °C, and only then diluting it with water to the use-concentration. In these challenge tests, boric acid esters, which are supplied as raw materials, and also numerous quite differently formulated easy-care coolants in practical use, were tested for their susceptibility to contamination and loadability on repeated contamination with microorganism. Among these easy-care coolants were also formulations that contained EP additives, for which a degree of antimicrobial effectiveness cannot be ruled out.

The tests were so designed that inoculation was carried out with a mixed population of bacteria, yeasts and moulds, and that contamination was carried out only with yeasts and moulds.

The preservatives used in these tests were benzisothiazolinone, alkylisothiazolinone, tris-hydroxy-nitromethane, Grotan F10 and Grotan Forte. Representative of the many challenge tests, the results of the tests with the following are presented here:

- boron-containing raw materials
- easy-care coolants with an oil content of between 20 and 50%
- easy-care coolants with a higher oil content and EP additives

[illegible]

- Descriptions such as “antibacterial” or “bacteriostatic” are closer descriptions of the properties of these formulations.
- It is not claimed that the raw materials and coolant formulations tested here is a complete list. They can only be regarded as representative of this substance class. It is therefore entirely possible that the antimicrobial properties can be improved with the use of other initial amines or the production of other structures.
- The bacteriostatic effect can only be achieved through exact adherence to the use-concentration, since the biological effect is only inhibition of growth (i.e. reversible damage); if the environmental conditions are altered by the inhibitory concentration not being reached, the bacteria can re-enter the growth phase.
- The danger of selection and adaptation is particularly great with products that in high concentrations only inhibit growth.

[illegible]

Table 5

	Oil content [%]	Boron content [%]	EP content [%]	pH-value 3% in water
Coolant C				
– With EP/A (= Zn/S/P compound)	60	0.89	0.82	9
– Without preservative				
Coolant D				
– Without EP/A = (UN/S/P compound)	70	0.83	< 0.03	9
– Without preservative				
Coolant E				
– With EP/S = (Zn/S/P compound)	68	0.80	0.70	9
– With preservative (not named)				
Coolant F				
– With EP/B = (Zn/S/P compound)	62	0.84	0.72	9
– Without preservative				

- Since the components of coolants can be rendered partially or totally unusable not only by microorganism, but by metabolic products and enzymes, from the technical, ecological and economic points of view it is advantageous to work with low numbers of microorganism.
- We do not consider microorganism counts of $> 10^6$ cfu/ml, which in some of the literature are said to be acceptable, to be appropriate. Coolant circulation plants can mostly be operated without technical problems with counts between 10^2 and 10^4 cfu/ml for bacteria. With counts of the same magnitude for yeasts and moulds, the conditions can certainly no longer be described as so favourable.
- It is known that e.g. large car manufacturers use preservatives even with easy-care coolants, with the aim of keeping the microorganism counts to $< 10^4$ cfu/ml, one of the particular aspects being industrial hygiene.
- From the results of our tests, supported by the comments above, it can be concluded that the use of preservatives when using easy-care coolants is sensible not only for technical, but also for ecological and economic reasons.

5.6.2.3 Preservatives

5.6.2.3.1 *Requirement profile for chemical preservatives.* A number of properties are required of chemical preservatives for use in water-mixed coolants, and these requirements should be met as far as possible.

Table 6

Boko with Bacteria(B) Moulds (M) Yeast (Y)		0 = Sterility control 1–12 = Number of inoculation cycles											– = free of microbial growth + = slight growth ++ = moderate growth +++ = massive growth	
Coolant	%	0	1	2	3	4	5	6	7	8	9	10	11	12
C	3	–	++ B	++ B	++ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M	+++ B,M	./.			
	5	–	+ B,M	+ B,M	+ B,M	++ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M	+++ B,M	./.		
D	3	–	++ BS	++ BS	++ BS	++ BS	++ BS	++ BM	+++ BS	+++ BS	+++ BS	./.		
	5	–	++ M,Y	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M
E	3	–	+ B,M	+ B,M	+ B,M	+ B,M	+ B,M	+ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M	+++ B,M	./.
	5	–	+M	+M	+M	+M	+M	+M	+M	+M	+M	+M	+M	+M
F	3	–	++ B	++ B	++ B	++ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M	+++ B,M	./.		
	5	–	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	++ B,M	+++ B,M	+++ B,M	+++ B,M

Table 7

Boko with		0 = Sterility control										– = free of microbial growth			
Moulds (M)		1–12 = Number of inoculation cycles										+ = slight growth			
Yeast (Y)												++ = moderate growth			
												+++ = massive growth			
Coolant	%	0	1	2	3	4	5	6	7	8	9	10	11	12	
C	3	–	++	++	++	++	++	+++	+++	+++					
	5	–	++	++	++	++	++	++	+++	+++	+++				
D	3	–	++	+++	+++	+++									
	5	–	++	++	+++	+++	+++								
E	3	–	++	++	++	++	++	++	++	++	+++	+++	+++		
	5	–	–	–	–	–	+	+	+	+	+	+	+	+	+
F	3	–	++	++	++	++	++	++	+++	+++	+++				
	5	–	++	++	++	++	++	++	++	++	+++	+++	+++		

- Broad spectrum of antimicrobial effect. In the recommended use-concentration the product should be effective against bacteria, yeasts and moulds.
- Rapid-effect. For several reasons the microbicidal effectiveness of a preservative is of particular importance. Microorganisms killed at an early stage no longer produce metabolic products, and thus e.g. enzymatic damage can largely be excluded. Further, a preservative is often used as a “fire engine”, i.e. it is not used until the microbial deterioration of the coolant is already far advanced and the properties of the lubricant may already be visibly changed: e.g. fall in pH, corrosion, increase in droplet size in the case of emulsions, unpleasant smell, etc. In such cases only a preservative with rapid microbicidal effectiveness can be of help.
- Lasting effect. This is a crucial economic factor, but almost always too much is expected of it because of unawareness of the fact that a preservative has to undergo a reaction with the microorganism in order to be effective. In this reaction it is obviously consumed. See chapter 2. Depending on the metalworking process, the preservative is also discharged to a greater or lesser degree.
- Loadability with organic substances. This means that the agent should if possible react only with microbe cells, and not with other components of the coolant or with organic dirt or the metals being worked or their ions.
- Good thermostability
- Good material compatibility, i.e. the preservative in the coolant must not cause any additional corrosion, for example, on the pieces being worked, and must not attack sealing materials or cause damage to paint.
- No change in the cooling and lubricant properties of the coolant
- The least possible effect on the pH value of the use solution
- Compatibility with the modules of ultrafiltration
- Ease of incorporation. Into coolant concentrates and balanced solubility in the water-mixed coolants.
- No disturbance of the environment
 - low odour
 - good skin tolerability
 - lowest possible toxicity
 - biodegradable
- High degree of economy

5.6.2.3.2 Substance classes. Since there is no one substance that can combine all the above mentioned properties, it is the task of professional preservative manufacturers to find practicable yet optimal compromise solutions by means of substance combinations and synergistic substance mixtures that permit use in different coolants for a wide variety of processes.

In the following, individual classes of agents from which preservatives can be formulated are considered in more detail.

5.6.2.3.2.1 *Aldehydes (II.2.)*. From the class of aldehydes, although the preservative effects of formaldehyde (II, 2.1.) (CAS N° 50-00-0), glutaraldehyde (II, 2.5.) (CAS N° 111-30-8) and benzaldehyde (CAS N° 100-52-7) are known, only the simplest, formaldehyde, in the form of its 30–40% aqueous solution has gained great practical importance. Its polymers, paraformaldehyde and trioxan, are irrelevant for preservation practice, as the formaldehyde is apparently too firmly bound. Formaldehyde has a balanced spectrum of effect against bacteria, but clear weaknesses against moulds.

As a result of the debate about formaldehyde and the resultant reaction of companies in no longer accepting formaldehyde as a preservative, the importance of glutaraldehyde is increasing to some extent.

5.6.2.3.2.2 *Formaldehyde donors*. Among the formaldehyde donors, the two main classes are

- O-formals (II, 3.1.)
- N-formals (II, 3.3.; 3.4.)

5.6.2.3.2.2.1 *O-formals*. The oxygen formals are obtained by reacting e.g. primary alcohols, glycols or glycol semi-ethers with formaldehyde. In almost all cases it is advisable to control the reaction so that it produces the so-called hemi-formals, as on account of their greater chemical stability the corresponding whole formals are either less effective or ineffective. The affinity of formaldehyde to alcohols (R-OH) is stronger than to H₂O (H-OH) in formalin. Hence the formaldehyde of the hemiformals from the preferred alcohols, such as e. g. benzyl alcohol (II, 1.4.), butyldiglycol (II, 1.16.) and ethylene glycol (II, 1.13.), is not as highly volatile as formaldehyde from formalin; thus the alcohol hemiformals are effective for longer.

Examples:

(Benzyloxy)methanol CAS N° 14548-60-8 (II, 3.1.2.)

(Ethylenedioxy)dimethanol CAS N° 3586-55-8 (II, 3.1.4.)

[2-(2-Butoxyethoxy)ethoxy]methanol CAS N° 260-097-2 (II, 3.1.5.)

With the oxygen formals, a class of compounds is available that can be used in coolants with relatively few problems, especially as they have virtually neutral pH values.

5.6.2.3.2.2.2 *N-formals*. Highly effective donor forms of formaldehyde are obtained by its reaction with amines or amides. Depending on the starting product and the reaction conditions, there are a large number of reaction possibilities. Thus we have such nitrogen formals as hexahydrotriazines, imidazolidines, oxazolidines, bisoxazolidines, amins and methylol compounds of amides.

The various substituted hexahydrotriazines result from condensation of primary alkylamines, primary hydroxyalkylamines and primary alkoxyalkylamines. These donor substances differ from formalin in their lesser volatility, longer effect, anticorrosive effect and better skin tolerability. However, the differences in these properties are quite considerable within the three derivative groups.

Products from primary alkylamines are quite economical (e.g. 1,3,5-triethylhexahydro-1,3,5-triazine (II, 3.3.19.) (CAS N° 7779-27-3), also quite effective against bacteria and moulds, but compared with the substances from hydroxyalkylamines (e.g. 1,3,5-tris(2-hydroxyethyl)hexahydro-1,3,5-triazine (II, 3.3.18) CAS N° 4719-04-4 and $\alpha, \alpha', \alpha''$ -trimethyl-1,3,5-triazine-1,3,5 (2H,4H,6H)-triethanol (II, 3.3.18a.) CAS N° 25254-50-6) are still more volatile and more aggressive to skin and mucous membranes. Due to the labelling requirement $\geq 0.1\%$ for 1,3,5-tris(2-hydroxyethyl)-hexahydro-1,3,5-triazine in the directive 67/548/EEC (R 43: May cause sensitisation by skin contact) there is an increased use of $\alpha, \alpha', \alpha''$ -trimethyl-1,3,5-triazine-1,3,5(2H,4H,6H)-triethanol in the market.

The compounds from the classes of the methylene-bisoxazolidines (e.g. 3,3'-methylenebis[5-methyloxazolidine] (II, 3.3.9.) CAS N° 66204-44-2), methylene-bis-oxazines (e.g. 3,3'-methylenebis-[tetrahydro-2H-1,3-oxazine] (II, 3.3.16.) CAS N° 63489-63-4) or amins (e.g. N,N'-methylene-bis-morpholine (II, 3.3.22.) CAS N° 5625-90-1) display better properties with regard to effectiveness against moulds, rapid microbicidal effectiveness, and temperature stability. Further advantages of these compounds are their solubility behaviour, as a result of which they can be used in anhydrous as well as water containing systems. Their aqueous dilutions are not as alkaline as e.g. those of the hexahydrotriazines, and are therefore less aggressive to the skin. The N,N'-methylene-bis-morpholine cannot be used in Germany. The TRGS 611 prohibits secondary amines and compounds which can release secondary amines.

1,3-bis(hydroxymethyl)urea (II, 3.4.3.) (CAS N° 140-95-4) is a formaldehyde donor that is inexpensive, but its antimicrobial effectiveness is considerably less than that of 2-chloro-N-(hydroxymethyl)acetamide (II, 3.4.1.) (CAS N° 2832-19-1), as the latter also has the effect of the activated halogen compound. The use of those halogenated compounds in coolants is limited; often corrosive effects have been observed.

5.6.2.3.2.3 *Nitro-derivatives*. Examples of condensation products from CH-acid compounds with formaldehyde are the reaction products tris-(hydroxymethyl)-nitromethane (tris-nitro) (II, 3.2.1.) and N-nitrobutyl-morpholine (II, 3.2.3.).

Examples:

Bronopol CAS N° 52-51-7 (II, 17.14.)

2-(Hydroxymethyl)-2-nitro-1,3-propanediol, CAS N° 126-11-4

4-(2-nitrobutyl)morpholine CAS N° 2224-44-4

2-(Hydroxymethyl)-2-nitro-1,3-propanediol has not gained any great market significance on account of its instability at pH values > 7.5 and lower effectiveness against moulds, and the 4-(2-nitrobutyl)morpholine on account of its greatly varying effectiveness depending on the structure of the coolant.

A further reason for the declining importance of these compounds is the fact that they are regarded as very effective nitrosating reagents. Their use was prohibited in 1993 by the TRGS 611.

5.6.2.3.2.4 Thiazoles. Viewed from the aspect of antimicrobial efficacy, these are very interesting compounds.

The good bacteriostatic and fungistatic properties of substances such as 2-(thiazole-4-yl)benzimidazole (II, 15.9) (CAS N° 148-79-8) or (benzothiazol-2-ylthio)methyl thiocyanate (II, 15.11.) (CAS N° 21564-17-0) were described in the literature more than thirty years ago. There have been repeated attempts to use these and other substances also in coolants. Questions of solubility, stability and the economically unfavourable effect/price ratios are obstacles to successful use as widely employable substances. On account of the lipophilic nature of these compounds one must not be surprised at very varying results.

1,2-Benzisothiazolinones and substituted isothiazolinone derivatives have been used for several years as components of preservatives.

However, the effect of these compounds is greatly dependent on the structure of the coolants.

5.6.2.3.2.5 Isothiazolones. 1, 2-Benzisothiazol-3(2H)-one (II, 15.6.) (CAS N° 2634-33-5) is often a failure in water-mixed coolants on account of the frequently inadequate availability of the substance resulting from poor solubility. It is mainly active against bacteria, but less active against *Pseudomonas* spec., which are one of the main contaminants in coolants.

The mixture of: 5-chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1) (CAS N° 55965-84-9) (II, 15.3.) is widely used for tank side preservation of water diluted coolants. The efficiency for a long term preservation is limited. The environment of a coolant contains many possibilities to inactivate these molecules.

For example, ammonia, amines, thiols and sulphites very strongly influence efficacy, especially of the mixture of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one, which can lead to loss of effect. Use in coolant concentrates is therefore ruled out from the outset.

Already alkaline pH values lead to a rapid break down of the 5-chloro-2-methyl-2H-isothiazol-3-one (II, 15.2.) (Figure 6), which is the main active compound in that mixture. It is c. 50-times more active than the 2-methyl-2H-isothiazol-3-one (II, 15.1.).

Handling the concentrated solution of 5-chloro-2-methylisothiazolin-3-one and 2-methyl-4-isothiazolin-3-one requires a number of protective measures because these substances are highly toxic and highly aggressive to skin and mucous membranes.

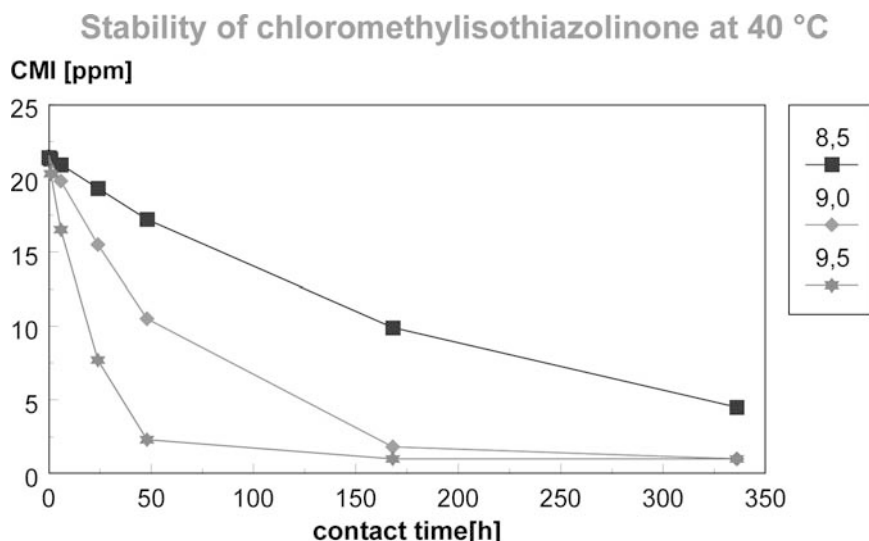


Figure 6

The use of these substances is therefore only recommended in formulated preservatives in which they are contained in a balanced formulation with other substances and suitable solvents. In diluted form these substances are then less dangerous to handle.

2-octyl-2H-isothiazol-3-one (II, 15.4.) (CAS N° 26530-20-1) is only effective as fungicide. Its stability in coolant concentrates is limited. Only special formulated products make it possible to add it to concentrates. Mostly it is applied tank side.

5.6.2.3.2.6 *Phenols* (II, 7.). Today, phenols play only a subordinate role in coolant preservation practice.

For ecological and toxicological reasons, polyhalogenated phenols such as tri-, tetra- or pentachlorophenol are no longer in use.

Like other microbicides phenols also dispose of advantages and disadvantages. One advantage is their rapid effect against bacteria and moulds, even in the presence of a relatively high organic load. Depending on the product to be preserved, however, in highly aqueous systems the phenols would have to be present in the form of their alkali salts, as otherwise, on account of their lipophilic properties, they would migrate into the non-aqueous phase and thus no longer be biologically available. On account of the possible shift in pH value, use in water-mixed coolants is limited, as at pH values considerably greater than 9 skin tolerability is greatly reduced.

Common to all phenols is a significant loss of their antimicrobial effect in the presence of non-ionogenic emulsifiers.

5.6.2.3.2.7 *Cationic compounds* (II, 18.1.). From the aspect of biocidal efficacy, cation-active compounds such as:

Didecyldimethylammonium-chloride (CAS N° 7173-51-5)

Quaternary ammonium compounds, benzyl-C8-18-alkyldimethyl, chlorides (CAS N° 63449-41-2)

would be appropriate. However, their marked lack of effect against *Pseudomonads* and their incompatibility with anionic surfactants prevent their use in practice.

5.6.2.3.2.8 *3-Iodo-2-propynyl butylcarbamate* (II, 11.1). 3-Iodo-2-propynyl butylcarbamate (CAS N° 55406-53-6) is a fungicidal agent that is increasingly being used in coolants. As a result of it also being approved as a cosmetic additive it is frequently evaluated positively by occupational physicians. Use both in coolant concentrates and for booster preservation is possible; however, when it is used in concentrates, the stability must be carefully tested.

5.6.2.3.2.8 *Amines* (II, 18.2.). Recent test results show that amines have both good corrosion protection properties and a microbicide-supporting effect, and, if the coolant concentrate is suitably formulated, remain effective even in anionic systems (e.g. 2-hexaminoethanol CAS N° 54596-69-9). As secondary amine it cannot be used according to TRGS 611 for the German market.

5.6.2.3.3 *Comparison of microbiological and application-technical parameters.* In Tables 8 and 9, evaluation criteria for microbiological efficacy and some application-technical properties are listed. The properties of the individual substance classes are evaluated on the basis of these parameters.

The properties specific to the substance classes obviously cannot be extrapolated to preparative preparations without limitation. As stated earlier, it is the task of preservative manufacturers to develop optimal preservatives for practical application on the basis of their specific knowledge of substances or substance classes for that particular application.

Table 8 Parameter

Substance class	Water solubility	Compatible components	Environmental influences	Toxicity (humans)
Alcohol-HCHO	☺	☹	☺	☺
Amide-HCHO	☺	☹	☺	☺
Amine-HCHO	☺	☹	☺	☺
Nitro derivatives	☹	☹	☹	☹
Octyl-isothiazolinone	☹	☹	☹	☹
Chloromethyl-isothiazolinone	☹	☹	☹	☹
Phenols	☹	☹	☹	☹
2-Mercapto-pyridin-N-oxide	☺	☹	☹	☹

☺ desirable

☹ limited

☹ unwelcome

Table 9 Microbiological efficacy

Substance class	Bacteria		Yeasts	Moulds	Immediate effect	Long-term effect
	Gram-positive	Gram-negative				
Alcohol-HCHO	☺	☺	☺	☺	☺	☺
Amide-HCHO	☺	☺	☺	☺	☹	☺
Amine-HCHO	☺	☺	☺	☺	☹	☺
Nitro derivatives	☺	☺	☺	☺	☹	☺
Octyl-isothiazolinone	☹	☹	☺	☺	☺	☺
Chloromethyl-isothiazolinone	☺	☺	☺	☺	☺	☹
Phenols	☺	☺	☺	☺	☺	☺
2-Mercapto-pyridin-N-oxide	☹	☹	☺	☺	☹	☺

☺ sufficient efficient
 ☹ restricted efficient
 ☹ moderate effective or ineffective

5.6.3 Summary

From what is stated here, from the literature currently available, and from our everyday work, customer care in matters of application technology, the following can be concluded:

- Users of water-mixed coolants must live with the fact that bacteria and fungi can grow and multiply in these use-solutions.
 - Heavily contaminated water-mixed coolants lead to technical and hygiene problems
 - microorganism are substantially involved in the deterioration of water-mixed coolants, e.g. as a result of metabolismisation of ingredients, for example through acid formation, and can render them unusable.
 - Skin irritation by microbial contaminated coolants – especially in cases of pre-damaged skin – cannot be ruled out; toxins, i.e. toxic breakdown products, are possibly influencing factors.
- The effects of microbial contamination on the coolants and the surrounding area in the factory can result in expensive problems.
- Physical treatment methods are too expensive, not sufficiently effective, or have not become established for other technical reasons. However, UV treatment of the mix water could be a useful measure for reducing microbial invasion.
- Even the easy-care coolants are not a general alternative. They display certain deficiencies in use; they are problematical with hard water; the concentrations must be adhered to very exactly, and in practice problems with yeasts and moulds repeatedly occur.
- Practice shows it every day: the microbial problems can currently only be avoided, or at least greatly reduced, by the use of chemical preservatives.
- Preservation, as a programme used effectively and in a controlled manner for controlling uninhibited microbial growth, helps us in the care of coolants and results in optimal practical conditions in the use of water-mixed coolants.

5.7 The microbial spoilage of polymer dispersions and its prevention

J. W. GILLATT

5.7.1 Introduction

Polymer dispersions, also referred to as polymer dispersions, latices, latex emulsions, latex dispersions or binders are used in an increasingly wide variety of applications including production of emulsion paints, adhesives, paper and textile coatings, non-woven fabrics and carpet making compounds.

In composition they are fine dispersions or suspensions of synthetic polymer particles ($0.1\ \mu$ to $5\text{--}6\ \mu$) in an aqueous stabilisation medium. The process of polymerisation can be of either of two types: addition polymerisation or condensation polymerisation. The main components of the aqueous stabilisation medium, apart from water, are colloids such as polyvinyl alcohol, cellulosic or starch materials and/or surfactants, being anionic, cationic or even non-ionic depending on the polymer type.

The pH of polymer dispersions varies greatly, being acidic in the case of some Ethylene Vinyl Acetate (EVA) and Poly Vinyl Acetate (PVA) types whilst some acrylic, styrene acrylic and styrene butadiene products can be relatively alkaline.

A common feature of most types of these polymer dispersions is that they are susceptible to spoilage by micro-organisms. Once growth occurs, a number of effects may be noticed including viscosity changes, production of gases and odours, colour changes and enzyme production with concomitant effects on manufactured end products.

The prevention of microbiological contamination by the incorporation of a preservative into the formulation requires a careful choice of biocide active agent, which is governed by the raw materials in the formulation, by the chemical environment and by legislative restrictions relevant to the end use of the manufactured product. Prevention in the supply chain requires an emphasis on the importance of manufacturing plant hygiene, especially during formulation processing, combined with the use of sampling and testing protocols in order to confirm the efficacy of the chosen preservative system. This involves closely monitoring each step in the chain from receipt of the raw materials through to the final use of the product and often even beyond this stage if the dry film is also susceptible to microbial attack.

5.7.2 The manufacture of polymer dispersions

Apart from water, the three main components required for the manufacture of polymer dispersions are (Gillatt, 1990):

- monomers
- initiators
- surfactants and/or colloids

5.7.2.1 Monomers

Polymerisation of one or more monomers is the basis of polymer dispersion manufacture. Many different monomers can be used depending on the end product required and its physical and chemical characteristics. Examples of monomers are listed in Table 1.

If a single monomer is reacted, the polymer obtained is termed a homopolymer. Many of the above monomers, however, can also react with each other, forming copolymers; varying the proportions of these comonomers affords an excellent means of controlling polymer properties.

Not all the monomers listed in Table 1 react together satisfactorily: thus vinyl acetate and styrene, ethylene and styrene, are examples of unfavourable pairs. Moreover, monomers without terminal unsaturation, e.g. the fumarates, and the related maleates, do not generally form homopolymers although readily forming copolymers with, for example, vinyl acetate.

The properties of the polymers are governed mainly by the monomer(s) on which they are based. All are thermoplastic, that is to say they soften progressively as their temperature is increased, but regain their original condition when cooled. Except when special reactive monomers are included, there is no cross-linking reaction, as occurs in thermosetting polymers, for example melamine-formaldehyde or urea-formaldehyde systems.

The hardness and softness of the polymer at a given temperature is predominantly determined by the monomer(s) employed. In polymers of identical make-up, differences in molecular weight are found to have a

Table 1 Monomers used in polymer dispersion manufacture

Monomer	Monomer name	Approximate molecular weight
HOOC-CH=CH_2	Acrylic acid	72
$\text{N}\equiv\text{C-CH=CH}_2$	Acrylonitrile	53
$\text{C}_4\text{H}_9\text{O-OC-CH=CH}_2$	Butyl acrylate	128
$\text{C}_2\text{H}_5\text{O-OC-CH=CH}_2$	Ethyl acrylate	100
$\text{CH}_2=\text{CH}_2$	Ethylene	28
$\text{CH}_3\text{O-OC-C(CH}_3)=\text{CH}_2$	Methyl methacrylate	100
$\text{HOCH}_2\text{-NH-OC-CH=CH}_2$	N-methylol acrylamide	102
$\text{C}_6\text{H}_5\text{-CH=CH}_2$	Styrene	120
$\text{CH}_3\text{-COO-CH=CH}_2$	Vinyl acetate	86
Cl-CH=CH_2	Vinyl chloride	62.5
$\text{C}_9\text{H}_{19}\text{-COO-CH=CH}_2$	Vinyl versatate	198

secondary effect, with higher molecular weight tending to reduce thermoplasticity slightly and lead to greater toughness and better resistance to solvents and water. Examples of monomers yielding hard generally brittle homopolymers requiring external plasticisers include: vinyl acetate, vinyl chloride, styrene, methyl methacrylate and acrylonitrile. Examples of monomers yielding soft internally plasticised homopolymers include: butyl acrylate, 2-ethylhexyl acrylate and the vinyl esters. Ethylene also acts as a plasticising comonomer with vinyl acetate and vinyl chloride but by a different mechanism.

All the polymers are fully saturated, i.e. they contain no residual double bonding. In consequence they are not prone to ageing effects of embrittlement and discoloration.

All monomers impart certain characteristic properties to polymers of which they form a part, but some have a particularly marked effect and can bring about significant changes in polymer behaviour even when present as only a few percent of the total monomer mix. As examples, acrylic, methacrylic acids and other unsaturated acids are used to give alkali-soluble copolymers in conjunction with methacrylic acid and acrylic esters. N-methyl acrylamide and other polyfunctional monomers produce reactive copolymers capable of secondary polymerisation or cross-linking by etherification or ionic bonding, usually after the watery portion of the emulsion has dried off.

5.7.2.2 Surfactants

Surfactants, by definition, are substances which lower the surface tension of liquids in which they are dissolved or the interfacial tension between two or more mutually immiscible phases. It has long been recognised that the most effective surface tension depressants contain highly water-attracting (hydrophilic) and highly water-repellent (hydrophobic) groups, joined together in the same molecule.

The most important hydrophilic (polar) groups found in surfactants are, for anionics, ionised sulphonate, sulphate, phosphate and carboxyl groups; for nonionics, polyoxyethylene chains and polyols; and for cationics, ionised tertiary or quaternary ammonium groups. The hydrophobic portion of the surfactant molecule usually consists of hydrocarbon radicals with more than eight carbon atoms, i.e. alkyl-aryl or long chain alkyl. Since the hydrophobic hydrocarbon groups have considerable affinity for oils and fats they have also been termed lipophilic or fat loving. Long polyoxypropylene chains, though less hydrophobic than hydrocarbons, nevertheless possess sufficient repellency to confer strong surface activity to compounds, wherein they are linked to hydrophilic groupings, such as polyoxyethylene chains. Thus a very versatile range of surfactants consists of block copolymers of ethylene oxide and propylene oxide in which the ratios and the molecular weights of the two different types of polyether chains are varied over a wide range. Table 2 gives some examples illustrating the chemical make-up found in typical commercial surfactants.

The behaviour of amphiphilic compounds in either aqueous or non-aqueous media is determined by the relative effectiveness of their hydrophilic groups and hydrophobic groups. In aqueous media the hydrophilic groups are strongly associated with the water molecules whilst the hydrophobic groups are repelled and tend to concentrate at the air-water interface. In the presence of both aqueous and oily phases the affinities of both

Table 2 Types of surfactant

Anionics	Nonionics
Stearate soaps	Polyethoxylated alkanol
Dodecyl sulphate	Polyethoxylated nonyl phenol
Dodecylbenzene sulphonate	Polyethoxylated polypropylene glycol (pluronic)
Diocetyl sulphosuccinate	Glyceryl monolaurate
Nonyl phenol polyether sulphate	
Dodecyl polyether phosphate	

groups can be satisfied by concentration of the surfactant molecules at the oil-water interface. The surfactant molecules will be orientated in such a way that the hydrophobic groups are associated with the non-aqueous phase while the hydrophilic groups are firmly anchored in the water layer.

If the interfacial area is small, it can only accommodate a small number of molecules. When, as usual, many more surfactant molecules than this are present, the majority cannot escape from the bulk liquid to the interface and the affinities of the hydrophilic and lipophilic groups must be satisfied by other means if thermodynamic stability is to be achieved. This again occurs by a process of orientation. In an aqueous medium the hydrophobic groups turn towards and associate with one another, forming in effect their own oil phase, surrounded by the hydrophilic groups turned outwards and anchored in the water. This type of internal association and orientation has been termed micelle formation. Micelles are usually spherical in shape. The 'escape mechanism' of micelle formation only becomes operative above a certain minimum surfactant concentration. This concentration has been termed the critical micelle concentration (CMC). CMCs vary from about $5 \times 10^{-2} \text{ mol l}^{-1}$ for the most hydrophilic to about $5 \times 10^{-4} \text{ mol l}^{-1}$ for the most hydrophobic types of surfactant. They are influenced by electrolytes, especially in the case of ionic surfactants, and also by other polar/non-polar chemical compounds such as alcohols, amides and, of course, other surfactants.

The most important consequence of micelle formation in the process of emulsion polymerisation is the solubilisation of organic compounds in aqueous media. Since the association of lipophilic groups inside a micelle leads to a formation of centres of attraction for organic compounds, it is possible to dissolve appreciably higher proportions of sparingly water-soluble monomers in micellar solutions than in water alone. Monomers, which are essentially non-polar in character, may be expected to dissolve only inside the hydrocarbon portion of the micelle. Compounds with polar groups can at least partially be accommodated in the water phase or at the micelle surface so that the requirements for the micelle dimensions are less critical.

The role played by surfactants in emulsion polymerisation (Bondy, 1966; Dunn, 1971) can best be demonstrated in a simple recipe involving only water, surfactant, monomer(s) and polymerisation initiator. When a sparingly water-soluble monomer is stirred into an aqueous surfactant solution it will be broken into droplets of varying size and give a more or less stable emulsion depending on the choice and quantity of surfactant present. If the surfactant concentration exceeds the CMC, some of the monomer will be solubilised in micelles. On the addition of a polymerisation initiator, and this almost invariably means a substance capable of producing free radicals, polymerisation will begin as soon as the initiator has been activated either thermally or chemically to yield free radicals (Elgood and Gilbekian, 1973).

The process of emulsion polymerisation begins when the free radicals derived from the, usually water-soluble, polymerisation initiator enter the monomer-saturated micelles where they find a sufficient number of solubilised molecules to start a rapid chain reaction (Elgood and Gilbekian, 1973). Each polymer radical first exhausts the monomer contained in the micelle and then captures additional supplies from 50 or more other micelles before the chain reaction is terminated. Some of the depleted micelles then break up and the released emulsifier molecules are adsorbed at the surface of the newly formed primary polymer particles (Dunn, 1971). The remainder are replenished by diffusion from the emulsified monomer droplets, which act essentially as reservoirs.

The formation of fresh polymer particles continues until all the emulsifier originally contained in the micelles is adsorbed at the larger polymer/water interface and the surfactant concentration has dropped below the CMC (Dunn, 1971). A new and different situation is thus created for the polymerisation, which would come to a halt if it could only proceed inside surfactant micelles. The growing polymer particle is, however, still very similar in general characteristics to a true (spherical) micelle. The chief difference is the composition and size of its lipophilic centre. On the outside there are still the layers of orientated surfactant molecules with their hydrophilic groups pointed towards the external water phase while the lipophilic groups are now associated, not with each other, but with the dispersed internal polymer phase. The conditions for monomer solubilisation are in fact greatly improved. Quite apart from the fact that the polymer particles have a far greater affinity for monomer molecules than the micelles ever had, there is now a considerable interfacial area available where interface solubilisation, akin to intramolecular solubilisation, can take place. It is this interface solubility of the monomers which is chiefly concerned in the second phase polymerisation, i.e. after the disappearance of the micelles. The loci of polymerisation have then shifted from the micelles to the polymer particles to which all the emulsified monomer is gradually transferred.

Now consider the case of polymerisation of monomers with appreciable water solubility and their copolymerisation with less water-soluble comonomers. Monomers such as vinyl acetate or methyl acrylate have sufficient water solubility to permit their rapid polymerisation in the water phase even in the absence or after the disappearance of surfactant micelles. New polymer particles can be formed as long as the monomer concentration in the water phase remains high enough. In most cases, there is strong monomer/polymer affinity so that more and more monomer will be extracted from the water phase. As polymer concentrations increase, polymerisation in the water phase will finally cease and with it the formation of new particles. Henceforth, conversion will proceed at the surface of the polymer/monomer particles in much the same way as in the case of water-insoluble monomers after the disappearance of the surfactant micelle.

At the end of the emulsion polymerisation, polymer particles will exist in the dispersed water phase with surfactant molecules adsorbed on the particle surface. It is usually considered that it is the hydrophobic portion of the surfactant that is adsorbed onto the surface while the hydrophilic portion goes into the water phase. The role of the surfactant is now to keep the system stable by preventing coalescence of the polymer particles in the dispersion. If particle coalescence is not prevented the settling of the coagulated particles can take place, giving a non-redispersible sediment.

For anionic surfactants, stabilisation of the polymer particles is achieved by the negative charge on the adsorbed surfactant molecules repelling neighbouring particles which are similarly charged. Stabilisation by this means is termed 'electrostatic stabilisation'. In effect, an energy barrier to particle coalescence is set up by charge-charge repulsion between particles. This energy barrier can be reduced by the addition of electrolyte, e.g. salts.

Non-ionic surfactants by definition carry no charge and with these surfactants stabilisation is mainly by volume reaction. Again the hydrophobic portion is adsorbed on the polymer surface while the hydrophilic portion, usually long ethylene oxide chains, extends into the water phase. Because of the volume occupied by these ethylene oxide chains the polymer particles cannot easily approach each other, i.e. there is an energy barrier to coalescence due to the spatial presence of the adsorbed surfactant. Stabilisation by this means is termed 'steric stabilisation'. The energy barrier to coalescence can be reduced by reducing the proportion of the ethylene oxide chains, e.g. either by salt addition or by heating the latex.

It should be noted that instead of stabilisation by surfactants the stabilisation of polymer particles may also be achieved by long chain water-soluble polymers. These are usually termed 'colloids' and typical examples are polyvinyl alcohol (Finch, 1973; Finch, 1992), hydroxyethyl cellulose and starch. Stabilisation is again by steric means. The main functions of colloids are:

- to give increased viscosity to the latex and to influence the structure and rheological properties, and
- to influence the application properties of the latex, e.g. give increased bond strength in adhesives.

Polyvinyl alcohol is supplied as a white free-flowing powder. Although the term 'polyvinyl alcohol' is used it is generally a copolymer of vinyl alcohol and vinyl acetate (Finch, 1973; Finch, 1992). The degree of hydrolysis represents the percentage number of moles of vinyl alcohol to the total number of moles present. Typical degrees of hydrolysis encountered in emulsion polymerisation are 80–100%. Quite different emulsion polymer properties and application results will be obtained using different hydrolysis grades of polyvinyl alcohol. The viscosity effects produced by polyvinyl alcohol will also depend very much on the molecular weight (MW), since higher molecular weight species produce higher viscosity latices (Farmer, 1992).

Polyvinyl alcohol is rarely used in paint systems as irreversible structures may be obtained with the gelling agent. For paints the structure may be achieved by using colloidal agents, such as hydroxyethyl cellulose.

5.7.2.3 *Initiators*

Initiators produce free radicals, which act on the monomer to form the polymer in aqueous conditions. Initiation may be thermal using a single chemical type or a redox system may be used (Elgood and Gilbekian, 1973). Redox systems are suitable for polymerisation at low temperatures. Some common initiator systems are listed in Table 3.

These redox chemicals may also be added at the end of the polymerisation process, as a finishing off stage (FOS), in order to get maximum polymerisation and to reduce residual unreacted monomer.

Other additives may be found in polymer dispersion systems, which contribute to their total composition. Examples of these include:

- Buffering agents – for pH control, e.g. sodium bicarbonate
- Antifoams – for preventing foaming
- Salts, e.g. sodium sulphate
- Metal catalysts, e.g. ferric chloride
- Preservatives – for protection against microbiological spoilage.

Table 3 Polymerisation initiators

Oxidising agents	Reducing agents
Potassium persulphate	Sodium formaldehyde sulphonylate
Sodium persulphate	Sodium metabisulphite
Ammonium persulphate	Sodium thiosulphite
t-Butyl hydroperoxide	Ascorbic acid
Cumene hydroperoxide	
Hydrogen peroxide	

5.7.2.4 The manufacturing process

The manufacture of polymer dispersions is a complex multi-stage process involving a large number of raw materials and several stages as the example below (Table 4), for the production of a styrene-acrylic dispersion shows (CRI, 2002).

Emulsion polymerisation is carried out in a reactor where temperatures may reach 90°C during the process. Thermally initiated emulsions are manufactured at temperatures between 70 and 90°C with redox initiation normally occurring at between 40 and 50°C. It is worthy of note that, although under such conditions the vegetative cells of most microorganisms will be destroyed, it is the author's experience that spores of bacteria and fungi can be occasionally found in emulsion samples taken directly from the reactor after polymerisation (Gillatt, 1990). However, it is the case that, in many instances, the temperatures and chemical environment during polymerisation is sufficient to destroy many microbial contaminants arising from raw materials.

From the reactor the emulsion is pumped through a network of pipework to the blenders, degassing tanks, open filtration systems and finally to the bulk storage systems (Figure 1). During this time the emulsion is cooling and possibly could become contaminated with microorganisms. For this reason a chemical biocide must be added to protect the dispersion from spoilage.

Biocides, although required in very small amounts, are often expensive and complex chemicals that are very labile to the hostile chemical environment of the polymerisation process and therefore their addition must be carefully controlled and monitored (Reeve, 1987; Conquer, 1993). The biocide is therefore added into the blender at a time determined when the temperature is low enough (<50°C) to prevent thermal degradation and when the free radicals have been consumed in the process (Conquer, 1993). This time is determined by a simple test to monitor the redox state of the emulsion. Under these conditions the biocide has the best chance of survival to allow it to perform its function as a long term preservative against microbial spoilage of the emulsion. The question of the stability of biocide active substances in the presence of redox chemicals is discussed later.

The chemical stability of the biocide and the accuracy of dosing of it into the emulsion can be determined in a number of ways, e.g. by high performance liquid chromatography (HPLC). This can be used as a quality control test method, carried out to confirm if the emulsion is adequately preserved and has a standard specification, along with other specifications of solids content, pH, viscosity, particle size distribution, residual monomer concentration, etc.

Another reason for checking the level of the biocide is for regulatory purposes where different emulsions, depending on their end use, have limits on their preservative type and content. Examples of these regulations include: US Food and Drug Administration (FDA) and German Bundesinstitut für Risikobewertung (BfR) regulations for direct and indirect food contact in food packaging applications for adhesives; BfR regulations for non-woven applications where binders are used in sensitive areas, e.g. feminine hygiene products, medical wipes and disposable nappies/diapers; and last but by no means least, areas where only food grade biocides can be

Table 4 Manufacture of a styrene-acrylic polymer dispersion

Material	Kg	Instructions	
Water	420.00	Heat to 85°C	A
Sodium bicarbonate	4.50		
Antarox CO630	5.70		
Water	155.00	Premix monomer emulsion	B
Abex AP-065	50.00	Add 55gm to reaction flask	
Antarox CO 897	6.00	Immediately add initial shot C	
Defoamer NS1	2.00	Commence monomer feed over 3 hrs and feed D over 3 hrs	
Styrene	440.00	concurrently with monomer emulsion.	
2-EHA	385.00	Hold temperature 80°C	
AA	13.50		
Water	15.00	Initial shot	C
APS	0.50	Use fresh APS	
Water	225.00	Feed with B over 3 hrs	D
APS	5.80		
Water	3.00	Add	E
APS	0.30	Hold 10 minutes	
t-BPO	0.60	Cool to 75°C and add	F
Water	12.00		
SFS	0.50	Add immediately	G
Water	4.00	Hold 30 minutes	
ACTICIDE® MV	3.60	Cool to 50°C and add	H
Water		Adjust pH and Solids	I
Ammonia			

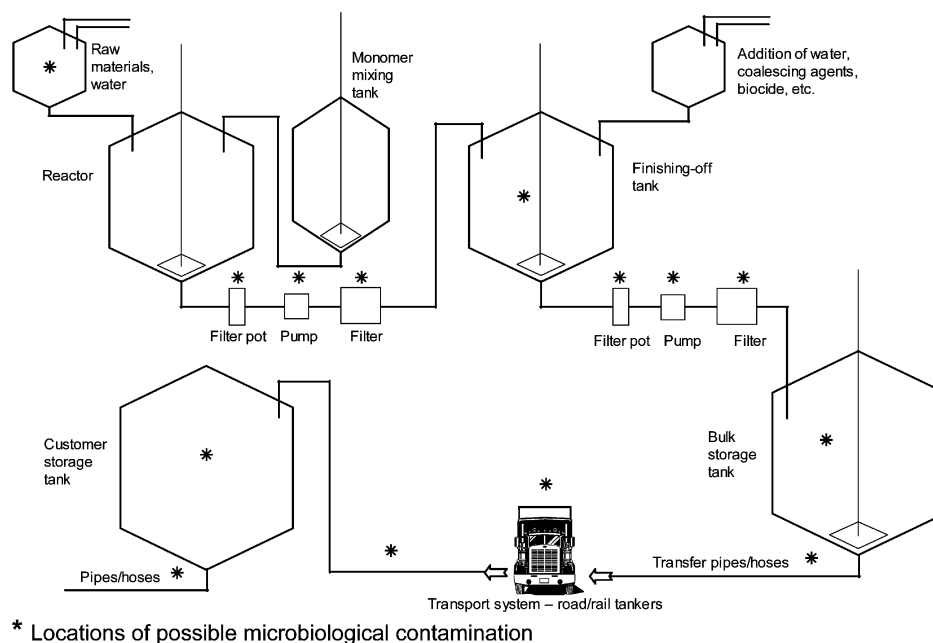


Figure 1 A schematic polymer dispersion plant.

can be incorporated, e.g. the adhesive used in cigarette manufacture for the seams in the paper and in the wax coatings for certain cheeses. In addition, the 28th Adaptation to Technical Progress (ATP) of The European Union's Commission Directive 67/548/EEC (EU, 2001) introduced labelling limits on a number of products, most notable the blend of 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and 2-methyl-4-isothiazolin-3-one (MIT) [II, 15.3.] such that formulations containing this combination at ≥ 15 ppm are required to display the Xi irritant symbol and the R 43 Risk Phrase ("May cause sensitisation by skin contact.").

5.7.3 Polymer dispersion types and their applications

There is a wide range of different types of synthetic polymer dispersions, incorporating many monomers in a variety of combinations (Farmer, 1994). Some examples of dispersion types include:

- Polyvinyl vinyl acetate (PVA) homopolymers
- Vinyl acetate/ethylene (VA/E) copolymers
- Vinyl acetate/vinyl chloride/ethylene (VA/E/VC) terpolymers
- Acrylic-containing (Ac) polymers
- Styrene/acrylic (Sty/Ac) polymers
- Vinyl acetate/vinyl versatate (VA/VeoVA) copolymers

If gaseous monomers such as ethylene or vinyl chloride are used, the production processes involve polymerisation at high pressures and the polymers formed are commonly referred to as 'pressure polymers', e.g. copolymers of VA/E or terpolymers of VA/E/VC or VA/E/2-EHA (2-ethylhexyl acrylate). Other non-gaseous monomers may be polymerised together to form polymers in low pressure systems and these polymers are commonly referred to as 'conventional polymers' or 'atmospheric polymers', e.g. VA homopolymers, Ac polymers of methylmethacrylate. Furthermore, the use of other functional monomeric units to give the polymer specific application properties, such as cross linking in cured textile fabric applications (e.g. *N*-methylol acrylamide, acrylamide and many others), are often employed and these polymers are commonly referred to as 'speciality polymers'. Common to all polymerisation reactions the processes are usually carried out in a batch-wise system, but continuous processes can also be employed.

Polymer dispersions are used in a large number of industries for the production of a rapidly increasing variety of routine and specialised products.

Elsom (1988) listed the following (Table 5) as major applications in which they are incorporated.

5.7.4 The microbiology of polymer dispersions

5.7.4.1 Components affecting susceptibility

The majority of polymer dispersions have a pH range between 3.5 and 9.5 with acrylic types in particular having a pH of greater than 8. It is often, therefore, the pH range and the fact that polymer dispersions are aqueous-based

Table 5 Applications of polymer dispersions

Industry	Examples of applications and uses
Adhesives	Packaging and converting, metal and plastic foil laminating, building, wood bonding, ceramic tiles fixing, bookbinding, cold seal, automotive assembly
Emulsion paints	Emulsion paint, from full gloss to matt for exterior or purely interior use – the emulsion acts as the binding medium, contributing durability and resistance to water, scrubbing etc
Textured coatings	Exterior and interior paints – the emulsion has the same function as in normal emulsion paints above
Industrial finishes	Wood and metal including anticorrosive finishes – special emulsions ensure requisite hardness, solvent resistance etc. in air drying of stoving finishes
Printing inks	Alkali-soluble emulsion polymers provide a reliable, consistent varnish base, particularly for flexographic inks
Building auxiliaries and cement additives	Emulsions with stability to high concentrations of ions, and with polymer components resisting the weakening action of water, oils, fats, etc. are widely used for general purpose bonding, and for upgrading the adhesion and resilience of renderings and floor toppings
Carpets	Polymer dispersions increase the tuft anchorage in woven carpets, without distracting from easy lay; in tufted carpet emulsions act as anchor coats for backing compounds, and in reinforcing and primary binders in backing
Non-woven fabrics	Emulsions provide the full range of cost performance properties meeting the various requirements of disposable and permanent non-woven fabrics, e.g. J-cloths, disposable nappies/diapers, feminine hygiene products
Textile finishings, printing and flocking	The versatility of emulsions covers the wide range of fabric finishing requirements, as well as the specific binding requirements for flocking printing, pigment binding etc
Glass fibre sizes and binders	Special emulsions bind glass strands and chopped strand mat for easy handling during lay-up
Paper and board coating	Emulsion binders for mineral coated stock enable good colour and printing properties
Wallpaper	Emulsions are used in all front coating operations, as well as duplexing and pre-pasting adhesives
Soil and mineral stabilisation	Surface binding of loose soil and mineral deposits by emulsions is widely undertaken to improve crop yields and reduce dust and similar environmental problems
Biological applications	Special emulsions with entrapped fungicides/mildewicides are used to coat seeds to prevent their decay prior to germination. Also, emulsions are used as cryo-preserving aids in the storage of harvested microbial cells

products, that determine their degree of susceptibility to the various classes of microorganisms. Acidic pH systems tend to favour the growth of yeasts and moulds, whereas neutral to alkaline pH systems tend to favour the growth of bacteria. Knowing this also helps when selecting and choosing the right biocide to preserve a particular polymer dispersion type.

Huddart (1983) reviewed the differences in pH between polymer dispersion types (Table 6) and commented on the effect that this was found to have on microbial growth.

Evertsen (1988) noted that products with pH 4–5 were rarely susceptible to growth although slight contamination was occasionally found in an acidic plasticised vinyl acetate homopolymer. He found alkaline acrylic and styrene acrylic types to be particularly susceptible.

Elsom (1988) found that polymers containing ethylene, especially ethylene/ethylene copolymers were particularly prone to infection and thought, therefore, that ethylene had a growth enhancing effect.

In the last decade, legislation and greater awareness of their toxicity have led to a general decrease of residual monomer concentrations in polymer dispersions. Such monomers often inhibit microbial growth and their reduction has resulted in products becoming more susceptible to attack (Conquer, 1993).

In addition, a number of other components will have a marked effect on the susceptibility of polymer dispersions to microbiological contamination. Jakubowski et al. (1992) studied the influence of raw materials on their susceptibility. By adding various of these to water at in-use concentrations, followed by inoculation with a variety of bacteria, yeasts and moulds isolated from contaminated products, they were able to show that many surfactants, defoamers and other additives were highly susceptible (Table 7).

Having considered the chemistry, manufacturing processes and applications of different types of polymer dispersions it is clearly indicated that their aqueous-based formulations provide, in the water phase, all the essential ingredients required for microorganisms to reproduce and flourish (Elsom, S.J., 1988; Evertsen, P.,

Table 6 pH of several polymer dispersion types

Acidic pH 3.5 – 6.5	Alkaline pH 8.0 – 9.5
Ethylene vinyl acetate	Acrylic
Polyvinyl acetate	Styrene acrylic
PVA/Acrylic	Styrene butadiene
PVA/Versatate	

Table 7 Susceptibility of polymer dispersion raw materials

Raw materials	Tested concn.	Susceptibility ^(a) to:		
		Bacteria	Yeasts	Moulds
Surfactants/wetting agents:				
Polyethoxyethanol	0.3%	—	—	—
Ethoxylated tetramethyl decinediol (30 moles)	0.3%	—	+	—
Ethoxylated tetramethyl decinediol (10 moles)	0.3%	—	+	—
Nonylphenoxypoly (ethyleneoxy) ethanol A	0.3%	—	—	—
Sodium salt of alkylaryl polyether sulphate	0.3%	—	—	—
Nonylphenoxypoly (ethyleneoxy) ethanol B	0.3%	—	—	—
Octylphenoxy polyethoxy ethanol	0.3%	—	+	—
Nonylphenoxypoly (ethyleneoxy) ethanol, wax	0.3%	—	—	—
Polyol emulsifier – liquid	0.3%	+	+	—
Polyol emulsifier – solid	0.3%	—	—	—
Defoamers:				
Proprietary liquid defoamer A	0.07%	—	—	+
Speciality formulated defoamer	0.05%	—	+	+
Proprietary liquid defoamer B	0.05%	—	+	—
Proprietary liquid defoamer C	0.07%	—	+	+
Thickeners:				
Hydroxyethyl cellulose thickener A	0.4%	+	+	—
Hydroxyethyl cellulose thickener B	0.16%	—	+	+
Hydroxyethyl cellulose thickener C	0.44%	+	+	—
Others:				
Polyvinyl alcohol A	0.65%	+	+	+
Polyvinyl alcohol B	1.44%	+	+	+

^(a) + = Susceptible to microbial infection: growth beyond 7 days.

— = Resistant to contamination following two successive inoculations with microorganisms.

1988; Cresswell, M.A., 1993; Cheesman, G.C.N. and Conquer, L., 1979). Although the limited amount of unreacted residual monomer present in many products may give some antimicrobial protection this is becoming less important as levels are reduced due to increasing legislation and awareness of environmental pollution. The trend is towards manufacturing and supplying emulsions free of organic emissions by employing various methods to remove or greatly reduce such components thereby producing products that can be claimed to be more 'environmentally friendly'.

There is some, albeit limited, evidence that certain monomers will actually aid in the spoilage of the dispersion itself, contradicting a widely held belief in the polymer dispersion and biocides industries that a residual level of monomer can give a biocidal effect (Cheesman, G.C.N. and Conquer, L., 1979). It is very much a question of concentrations.

Microbial attack of vinyl chloride has been shown to occur (Nelson, Y.M. and Jewell, W.J., 1993) and still further it is known that vinyl acetate breaks down to give acetaldehyde and acetate (Nieder, M., Sunarko, B. and Meyer, O., 1990), both metabolites being microbial nutrients at the concentrations found in typical polymer dispersions, where free vinyl acetate concentrations can be in the order of 0.1%. At higher levels, as may have been found some years ago, acetaldehyde would have been present at preservative concentrations.

Free acrylate monomers may be available to some types of bacteria, especially *Pseudomonads*. The precise mechanism by which they are utilised is not clearly demonstrated but probably occurs by hydrolysis of the monomer to acrylate and the alcohol.

In the past, the residual monomer was regarded as an essential component of the emulsion system to afford some degree of antimicrobial protection, although it was only ever adequate for normal storage of emulsions in closed containers. For prolonged storage, or in vessels that are not well closed, the protective action from residual monomer will decline as it slowly evaporates, requiring the use of additional preservatives. Moreover, the protective effects from the residual monomer are not sufficient to withstand the additional populations of microorganisms likely to be introduced with pigments, fillers etc., or withstand heightened microbial activity in the presence of additional colloids.

As noted by Jakubowski et al. (1982) above, the stabilising colloids are also a good source of microbial nutrition. Some surfactants contain long chain fatty acids and are, in consequence, fairly easily degraded. Substituted celluloses, which are commonly used as stabilisers, may be attacked by microorganisms, the amount of nutrient available from this source depending on the type and degree of substitution. Celluloses may also provide nutrients from their catalytic chemical breakdown throughout the reaction. The degradation of cellulosic materials by a variety of microorganisms has been reported abundantly in the scientific literature over the past 100 years, as detailed in two reviews on the subject by Doelle (1984) and Enari (1983).

The polymer component itself is virtually unaffected by microorganisms, but the degradative effect on the water phase can lead to spoilage of the emulsion manifested by the production of a number of undesirable effects.

5.7.4.2 Causative microorganisms

The majority of bacteria, yeasts and mould microfungi commonly living in the environment can be found in samples of polymer dispersions, as well as in formulated products containing them. Microorganisms are extremely versatile in their modes of nutrition, many organisms having very simple requirements needing only an organic carbon source and inorganic source of nitrogen, phosphorus and sulphur. Some individual genera are capable of utilising a wide variety of compounds, e.g. *Pseudomonas spp.* which can utilise many hundreds of organic substrates, ranging from simple compounds such as acetate to complex organic chemicals used as disinfectants in hospitals, e.g. phenols and organohalogen compounds frequently used as ingredients in the formulation of biocides.

Various chemical, physical and environmental factors, noted above, will determine the types of microorganism that can be isolated from different synthetic polymer dispersions and there are a number of literature summaries of organisms shown to be major contaminants of a range of these products (Table 8).

Furthermore, collaborative testing was carried out by participants in the Polymer Dispersion Group of the International Biodeterioration Research Group (IBRG) in which the viability in polymer dispersions of 175 microbial species was evaluated (Gillatt, 1995) (Table 9). Sixteen representative organisms were selected from

Table 8 Microorganisms isolated from polymer dispersions

	Elsom (1988)	Carter (1982)	Evertsen (1988)	Jakubowski et al. (1982)
Bacteria				
<i>Achromobacter sp.</i>	X	X		X
<i>Acinetobacter sp.</i>			X	
<i>Alcaligenes sp.</i>	X	X		
<i>Arthrobacter sp.</i>	X			
<i>Bacillus sp.</i>		X	X	X
<i>Citrobacter freundii</i>			X	X
<i>Corynebacterium</i>	X			
<i>Enterobacter cloacae</i>		X		
<i>Enterobacter sp.</i>	X	X		X
<i>Escherichia coli</i>		X	X	
<i>Flavobacterium sp.</i>		X		
<i>Klebsiella pneumoniae</i>				X
<i>Lactobacillus sp.</i>		X		
<i>Micrococcus sp.</i>		X	X	
<i>Proteus mirabilis</i>			X	
<i>Proteus rettgeri</i>				X
<i>Proteus sp.</i>		X		
<i>Proteus vulgaris</i>			X	
<i>Providencia alcalifaciens</i>				X
<i>Providencia rettgeri</i>				X
<i>Pseudomonas aeruginosa</i>			X	X
<i>Pseudomonas cepacia</i>				X
<i>Pseudomonas fluorescens</i>			X	X
<i>Pseudomonas maltophilia</i>				X
<i>Pseudomonas putida</i>			X	
<i>Pseudomonas sp.</i>	X	X		X
<i>Sarcina luteus</i>			X	
<i>Serratia marcescens</i>		X		
Moulds and Yeasts				
<i>Aureobasidium sp.</i>		X		
<i>Alternaria sp.</i>			X	
<i>Aspergillus sp.</i>		X	X	
<i>Bimorphic fungus</i>				X
<i>Candida boidinii</i>		X	X	
<i>Cladosporium sp.</i>		X	X	
<i>Filamentous fungus</i>				X
<i>Fusarium sp.</i>		X	X	
<i>Geotrichum candidum</i>	X			
<i>Geotrichum sp.</i>		X		X
<i>Penicillium sp.</i>	X		X	
<i>Pichia sp.</i>				
<i>Rhodotorula rubra</i>			X	
<i>Saccharomyces sp.</i>	X	X		X
<i>Torula sp.</i>		X		X
<i>Torulopsis sp.</i>	X			

Table 9 Polymer dispersion microorganisms, IBRG study

Organism	Number of species	Of which:
Bacteria		
<i>Pseudomonas</i>	30	12 were <i>Pseudomonas aeruginosa</i> 6 were <i>Pseudomonas putida</i> 5 were <i>Pseudomonas fluorescens</i> 5 were <i>Pseudomonas stutzeri</i>
<i>Escherichia</i>	11	All were <i>Escherichia coli</i>
<i>Alcaligenes</i>	11	6 were <i>Alcaligenes faecalis</i>
<i>Proteus</i>	9	6 were <i>Proteus vulgaris</i> 2 were <i>Proteus morganii</i>
<i>Flavobacterium</i>	6	Various species
<i>Klebsiella</i>	5	3 were <i>Klebsiella pneumoniae</i>
<i>Micrococcus</i>	5	4 were <i>Micrococcus luteus</i>
Moulds:		
<i>Aspergillus</i>	10	5 were <i>Aspergillus niger</i> 2 were <i>Aspergillus oryzae</i>
<i>Geotrichum</i>	7	5 were <i>Geotrichum candidum</i>
<i>Penicillium</i>	7	2 were <i>Penicillium ochrochloron</i>
Yeasts:		
<i>Candida</i>	7	3 were <i>Candida albicans</i> 2 were <i>Candida valida</i>
<i>Rhodotorula</i>	4	2 were <i>Rhodotorula glutinis</i> 2 were <i>Rhodotorula rubra</i>
<i>Saccharomyces</i>	2	Both were <i>Saccharomyces cerevisiae</i>

these and were found to grow or survive in neutral and alkaline dispersion formulations. These later formed the basis of the draft IBRG standard polymer dispersion challenge test (IBRG, 2001).

Notwithstanding that a huge range of microorganisms can and have been isolated from contaminated polymer dispersions, it is the author's experience that the most common spoilage bacteria isolated from polymer dispersions are non-lactose fermenting, Gram negative rods. The most predominant genera are the Pseudomonads, organisms with very powerful biodeteriogenic capacities. Other genera are also frequently isolated in specific dispersion types, including, occasionally, Gram positive organisms.

It is probable that, as strains of both Gram negative and Gram positive bacteria are capable of polymer degradation, the type of microorganism responsible for the breakdown of particular polymers will be dependent upon the polymer constituents and the accessibility of each microorganism to the polymer (Hesketh et al., 1995a, Hesketh et al., 1995b, Hesketh et al., 1994). In either case, it is imperative that the polymer dispersion manufacturer uses a biocide active against both Gram negative and Gram positive bacteria.

There have been recent, frequent experiences of acidic polymer dispersions, especially those containing polyvinyl alcohol (PVOH) being contaminated with slow growing, biocide tolerant, *Gluconoacetobacter* species, especially *G. liquefaciens*.

Elsom (1988) and the author (1990) have also commonly found anaerobic sulphate reducing bacteria (SRBs) in several polymer dispersions of varying composition. These microorganisms tend to occur in systems that have been previously contaminated with numerous aerobic species and are generally regarded as the last organisms to arise in a microbial ecological succession. SRBs tend to occur at the bottom of bulk storage vessels/tanks, cause blackening of the emulsion and are responsible for foul sulphurous odours due to the terminal electron acceptor in their respiratory chain being hydrogen sulphide.

Oppermann and Goll (1984) also investigated the incidence of anaerobic microorganisms and, in their study of seventy contaminated samples, found a wide variety of species of which *Bacteroides*, *Clostridium*, *Desulphovibrio* and *Bifidobacterium* were the most common.

Bizarrely, the author recently experienced a polymer dispersion in a manufacturer's storage tank contaminated with a species, so far unidentified, of blue-green algae (Figure 2).

5.7.4.3 The effects of microbial contamination

If microbial spoilage does occur, the consequences can be severe since it can cause deterioration of not only the polymer dispersion itself but may have concomitant effects on products into which the dispersion is formulated, rendering them unsuitable for their intended purpose. The effects of microbiological spoilage of these aqueous-based synthetic products may often go unnoticed until it is too late to take remedial action. As such, if substrates are contaminated and the microorganisms and their by-products, e.g. extracellular enzymes, are not inactivated, or at least their growth and activity inhibited heavily, they are able to decompose the product's ingredients, a consequence which manufacturers or consumers will realise when one or more of the following unacceptable symptoms are noticed (Elsom, 1988; Evertsen, 1988; Cresswell, 1993) (Table 10).



Figure 2 Blue-green algae on a polymer dispersion.

5.7.4.3.1 Effects on products. The effects of microbial contamination on polymer dispersions themselves are listed as items 1–8 in Table 10 with other effects on production plants and the environment.

5.7.4.3.1.1 Viscosity changes. Viscosity loss of the emulsion and of formulated products, by breakdown of colloids and surfactant-stabilising systems of emulsions by microbial attack or by extracellular enzymes, are one of the most commonly noticed effects of contamination. Since the viscosity and rheology are fundamental to the product's applicability characteristics, a contaminated product can be unusable. Another consequence of viscosity instability is that phasing may be induced leading to a non-homogeneous product with thinner liquid on the top and thicker material on the bottom. This phenomenon is often termed 'syneresis' and is the result of settling and destabilisation of the product after effects to its rheological and viscosity properties.

The author has also, unusually, seen examples of polymer dispersions where an increase in viscosity has occurred as a result of microbial infection. The mechanism of this has not been investigated but is thought to be due to microbial acid production (see below), causing a reduction in pH and flocculation of one or more of the dispersion's ingredients.

5.7.4.3.1.2 pH changes. pH changes, most usually a reduction in pH, can arise due to fermentative metabolic processes of microbial cells, breaking down colloidal components of the product. The most common metabolic products are simple organic acids causing a reduction in pH by one or more full pH units. As noted above, this acid production can lead to product destabilisation as well as possible corrosion to storage vessels and containers.

Occasionally an increase in pH has been noted and this is thought to be as a result of secondary infection with one of the ammonia-producing bacteria, e.g. *Brevibacterium sp.*, although there is no evidence that this is the case.

5.7.4.3.1.3 Gas formation. Most commonly, any initial gas production will be of carbon dioxide, resulting principally from the metabolism of fermentative bacteria breaking down colloidal thickeners, e.g. cellulose to

Table 10 Effects of microbiological contamination

1	Viscosity changes
2	pH changes
3	Gas formation
4	Malodour
5	Colour changes
6	Visible surface growth
7	Changes in other properties
8	Enzyme production
9	Effects on production plants, e.g. biofilm formation, corrosion
10	Environmental effects

glucose, which is then fermented to yield acid and carbon dioxide. As such gas this gas production is often associated with a reduction in pH, noted above. Such gas production is not usually noticed during production but can cause distortion and even splitting or 'lid popping' of containers. Gas formation is often accompanied by the production of unpleasant odours.

5.7.4.3.1.4 Malodours. The most common malodour associated with microbial spoilage is the sulphurous 'bad egg' odour produced by the sulphate-reducing bacteria, e.g. *Desulphovibrio desulphuricans*, which, under anaerobic conditions, can utilise oxygen from sulphates leading to hydrogen sulphide production. This type of odour production is often combined with a noticeable blackening in the colour of the product. In itself this odour can be unpleasant for the user but potentially more serious is the possibility that the odour will reappear in a formulation in which the dispersion is used, e.g. an adhesive or paint. A contaminated adhesive used for food packaging, for example, could lead to product rejection by the final consumer. Other unpleasant rancid or musty odours are derived from various microbial by-products such as butyric acid. Mottram et al. (1984) described the production of an 'intense obnoxious musty odour akin to dirty drains' and found this to be caused by an unidentified *Pseudomonas*-like organism able to produce the metabolite 1,6-dimethyl-3-methoxypyrazine.

5.7.4.3.1.5 Colour changes. The most common colour change seen in polymer dispersions is as a result of the formation of insoluble sulphides such as iron sulphide produced by the sulphate reducing bacteria. Most frequently this is seen at the bottom of containers where the oxygen concentration is lowest. However, the author has seen polymer dispersions in closed containers where blackening has commenced from the top and spread downwards, presumably as a result of aerobic bacteria, more active on the surface, first reducing the oxygen concentration there.

Other microorganisms, particularly the pink yeasts such as *Rhodotorula rubra* and *Sporobolomyces roseus*, and pigmented mould fungi can produce other discolorations. This is often visualised in the appearance of an oily yellow film on the product's surface.

5.7.4.3.1.6 Visible surface growth. Surface growth of aerobic mould microfungi on the surface of products commonly leads to visible surface disfiguration. This is particularly noticeable in the case of higher viscosity acidic formulations, especially those thickened with cellulosic derivatives. However, in these cases, testing often reveals that only the surface is contaminated and the bulk of the product below is free from microorganisms.

Occasionally, alkaline products become contaminated with visible surface bacterial colonies, although this has only been noticed in laboratory test samples.

Mention has already been made of a polymer dispersion where the surface in a storage tank was severely contaminated with a visible growth of a blue-green algae. But it is believed that this one example is unique.

5.7.4.3.1.7 Changes in other properties. Several other changes can occur in the properties of polymer dispersions arising from microbial infection. Some of these can particularly affect formulated products in which the dispersion is used. Microbial growth, resulting in destabilisation and viscosity loss of the product as well as attack on the polymer particles, can lead to a reduction in the average molecular weight of the polymer. In this case, the use of such a product in, for example, an adhesive, would affect that product's bond strength to a level unacceptably lower than that of a corresponding non-contaminated adhesive. In practice, this is unlikely to be frequently seen since, if deterioration has progressed to this stage, the contamination is likely to be detected before the dispersion is used.

5.7.4.3.1.8 Enzyme production. Enzymes, e.g. cellulases and amylases, are commonly produced by bacteria and mould fungi as a means of their utilising complex carbohydrates. Once the long chain polymeric units are broken down to oligomers and, eventually, monomers the thickening agents are no longer able to carry out their prime function and a loss in viscosity may be seen. Cellulases are effective at concentrations as low as 10^{-5} enzyme units per ml in producing this effect. An enzyme affected product cannot easily be recovered because, even though the causative organisms can be killed with by a further biocide addition, the enzymes will remain active. This was demonstrated by (Figure 3) by measuring the viscosity of a contaminated product in which the microorganisms had been killed by a high concentration of 5-chloro-2-methyl-4-isothiazolin-3-one plus 2-methyl-4-isothiazolin-3-one (CIT/MIT). It was seen to decline dramatically over 42 days. When rethickened with the same cellulosic thickener as originally used, the viscosity was seen to once again decline, even in the absence of viable microorganisms (Gillatt, 1992).

Once a polymer dispersion or adhesive has been used in a formulated product, microbial growth can still take place either in the liquid phase, e.g. in paints and inks or, especially in high moisture environments, on dried materials after application, e.g. ceramic tile adhesives and paint films. To prevent this, biocides need to also be used in such end products, i.e. wet-state preservatives in liquid formulations and dry-film fungicides/algicides in products susceptible to such attack or infection (Ludwig, L.E., 1974; Springle, W.R., 1990; Paulus, W., 1992;

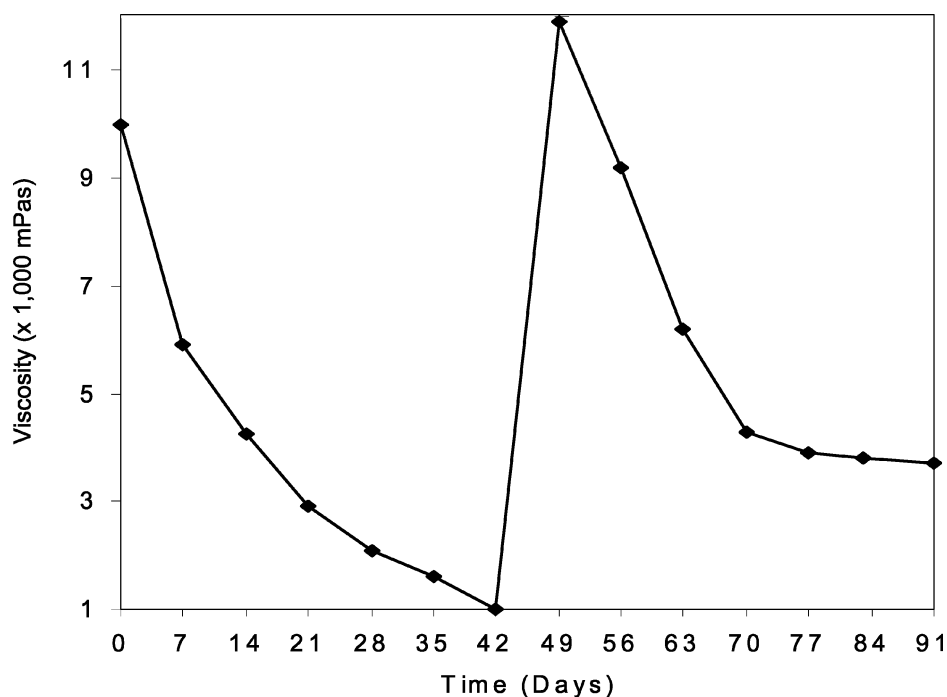


Figure 3 Viscosity loss in a cellulosic thickened product.

Paulus, W. (ed.), 1992; Miller, G.A. and Lovegrove, T., 1980). However, dry-film preservatives are not generally necessary in most aqueous-based adhesives and polymer dispersions.

In general, for end uses where a polymer dispersion will be in contact with an environment conducive to the propagation of microbial infestation, a contaminated product cannot be ignored as a possible source of further contamination. All the effects detailed above, resulting from microbial spoilage of polymer dispersions, must be fully investigated in terms of product quality complaints because they can lead to significant economic loss to the manufacturer if products reach the consumer in a deteriorated condition.

5.7.4.3.2 Effect on production plants. In addition to the effects on the products themselves, microbial infection of polymer dispersions causes a number of other problems in the plants in which they are manufactured. Biofilm formation on the inside of pipework, hoses, mixing and storage vessels can cause blockages and filtration problems and may lead to the development of foci of anaerobic sulphate reducing bacteria colonisation. This, in turn, can give rise to actively anodic sites causing often severe localised pitting corrosion. In this situation the microorganisms not only constitute a deposit problem in themselves, but also entrap other materials, especially fillers and other particulate matter, which might normally have remained in the aqueous phase of the dispersion; thus deposit formation increases and, inevitably, more corrosion results (Beecher et al. 1995).

Such spoilage outbreaks in a manufacturing plant can range from simple situations that are relatively easy to eradicate with the use of sanitation measures combined with the use of effective biocides, to more complex situations if spoilage is allowed to go unchecked for prolonged periods. Then significant economic loss to the company is realised, ranging from that caused by long periods of downtime whilst the problem is resolved to the need for major capital investments to replace pipework and tanks or other corroded metalwork in the plant.

5.7.4.3.3 Environmental effects. As with plant related problems, the effects of microbial contamination on the environment both inside and outwith the production area are several and varied. One most noticeable effect is that of foul odour with potential associated health risks.

These effects are less apparent to the manufacturer than to the end user of the polymer dispersion, but they must not be overlooked. Both the foul odours, particularly from hydrogen sulphide, and the actual presence of microbial spores can have a serious effect on man. Hydrogen sulphide levels in contaminated products have never been shown to reach toxic concentrations but even very small amounts can render a product unsaleable. In both of these examples there are strict guidelines on occupational exposure. In the case of microbial spores, over-exposure can lead to respiratory disorders and asthmatic symptoms.

Another environmental effect that cannot be overlooked is the problem of disposal of possibly large volumes of polymer dispersion should such quantities become so contaminated that they cannot be used. Such disposal is difficult and expensive, involving the splitting of the emulsion, separation of the aqueous and non-aqueous phases, treatment of these and safe and responsible disposal.

5.7.4.3.4 Effect on the manufacturer's business. It must not be forgotten that we exist in a commercial world. The effect of microbial contamination on product quality can severely damage a manufacturer's reputation in the market to the extent that they may lose the business in part or totally, or even suffer high financial claims for the end-user's losses, which may be settled in or out of court. All this can lead to interrupted work in production, additional time which must be put in to rectify the quality incident and disposal costs. Unwanted microorganisms are expensive!

5.7.4.4 Sources of microbial contamination

As previously mentioned, polymer dispersions when first produced are infrequently contaminated save, occasionally, with the spores of bacteria and mould fungi. It can therefore be assumed that the majority of spoilage microorganisms must gain access subsequent to the completion of the polymerisation reaction. When a major outbreak of contamination does occur, initial responses tend to centre on methods of wiping out the immediate problem. Identification of the source of contamination is considered as an afterthought, if at all.

The sources of contamination are limited and many are generally within the control of the manufacturer (Gillatt, 1993) (Table 11). Realistic action taken to identify these sources, quantify the potential risk from each and reduce or eliminate the degree of contamination at that point can save a great deal of time and expense later.

5.7.4.4.1 Airborne contamination. Microorganisms do not just appear from thin air; or do they? Well, of course, the air is a rich source of microorganisms but this is often overlooked because they cannot be seen with the naked eye. It is accepted that the human race lives in equilibrium with a wide spectrum of microorganisms in the environment. A dusty atmosphere from building operations, animal pollution from farms, stables etc., proximity to orchards, gardens, etc. leading to yeast and mould contamination, are typical hazards for any factory making aqueous-based products that are vulnerable to microbiological spoilage. In the summer, wind blown dusts, rich in soil microorganisms, enter the factory through curtain doors left open for cooling and cause contamination, especially in the open parts of the plant. Diehl (1986(a) and 1986(b)) reported that air can contain 500–2,000 microorganisms per m³ which may include spores of some polymer dispersion spoilage species.

Many parts of polymer dispersion manufacturing plants (Figure 1) tend to be open to the atmosphere. In many locations, due to the nature of the chemicals used and the normally open filtration and sieving processes, are critical points at which microbial contamination may occur.

5.7.4.4.2 Raw materials.

5.7.4.4.2.1 Water. Water is the main raw material in the majority of aqueous formulations, including polymer dispersions. However, the process water is unlikely to be a primary source of microbial contamination, as any microorganisms present tend to be destroyed in the reactor. Nevertheless, as mentioned above, some spore-forming microorganisms and yeasts can survive the polymerisation process and these have been isolated from fresh polymer dispersions soon after production. It is therefore necessary to ensure that process water is treated before its use.

Process water may originate from a number of sources including:

- The mains system
- Boreholes and wells
- Rivers and canals

In many countries it is compulsory to have a so-called break tank, or storage tank, between the actual water supply and the manufacturing plant. Such storage tanks are common foci of contamination, often in the form of adhered biofilm on the tank walls and in associated pipework (Figure 4).

The microbiological quality of process water will vary according to its source and is often treated before use. Chlorination alone may not be totally effective, especially if organic matter is present, when residual

Table 11 Sources of microbial contamination

Source	Control
The air	
Raw materials (including process water)	
Environment	
Manufacturing equipment	Manufacturer can control (mostly)
Operators	
Packaging materials	
In use	Manufacturer cannot control



Figure 4 Bacterial biofilm inside a water storage tank.

contamination may remain. As suggested by Duddridge (1987), even supposedly good quality town tap water may contain bacteria, for example *Pseudomonas* species at up to 1,000 cells per ml.

Process water originating from wells, boreholes, rivers and canals are often much more highly contaminated than the mains supply and need to be treated appropriately. Filtration is required to remove large particulate organic matter and the filter pads used must be themselves backwashed and treated from time to time. Residual microorganisms can then be treated with a biocide such as chlorine, chlorine dioxide, ozone or another rapid acting substance. Some effect may be achieved by introducing an inline ultraviolet treatment system, although the author has seen bacterial biofilm growing on the inner surface of the quartz tubes when they have not been properly maintained.

Some polymer dispersion manufacturing processes require the use of deionised water. Water, with even a low concentration of microorganisms, passing through an ion exchange column can lead to the formation of a biofilm around the ion exchange resin particles, resulting in the continual low level inoculation of the water passing through. As with filters, ion exchange columns must be regularly backwashed and treated to prevent such biofilm build up.

However, assuming adequate control measures are taken to ensure the supply water contains as few microorganisms as possible, the most likely source of contamination from water will be from untreated residual wash water left in mixing vessels, hoses, etc.

5.7.4.4.2 Other raw materials. Most of the initial raw materials are either likely to have a very low level of contamination or any that is present is likely to be killed during the reaction process. Therefore, the majority of raw material linked contamination is likely to come about as the result of adding materials e.g. thickening agents such as polyvinyl alcohol, which may be contaminated with microorganisms, at the end of the manufacturing process. Nichols (1989) suggested that raw materials were probably the single biggest potential contamination source. Malcolm (1980) arbitrarily classed raw materials into three groups: non-aqueous synthetics, aqueous synthetics and natural.

Some of the powdered raw materials, especially those originating from natural sources, such as extenders/fillers can often be contaminated with dormant spores of bacteria and fungi which can germinate once an aqueous environment is provided.

Liquid raw materials such as defoamers, surfactants, starch solutions and hydroxyethyl cellulose solutions may themselves be susceptible to microbial attack and, unless carefully manufactured and protected with biocides, can also introduce contamination into the product.

5.7.4.4.3 The manufacturing plant. The manufacturing plant itself is often one of the main sources of microbiological contamination and this can arise from a number of points.

5.7.4.4.3.1 The manufacturing process. Increased attention to quality control, and the introduction of ISO 9000 quality systems, has led to more detailed examination of the raw materials used. However, even if

raw materials are free from infection before use, the method of manufacture itself may result in contamination of the end product. The addition of biocide to a product as soon as practicably possible after the polymerisation process is therefore recommended. Ideally, it should be added to the product as soon as it leaves the reactor on pump out to the blender. Incorporation of the biocide can be made with the dilution water required to bring the product into its solids content specification. It must also be noted that if thickening solutions of colloids are employed post-manufacture these also should be protected with a biocide. However, as will be noted below, care must be taken to ensure that the biocide added is stable at the conditions of temperature, pH and redox potential prevailing at the addition point.

5.7.4.4.3.2 Plant design. The design of modern manufacturing plants is often extremely complex and it is said that the greater the complexity the greater the skill of the chemical engineer. If the production unit is built in such a way that it is difficult to physically keep clean then there is a greater likelihood of contamination foci building up and causing product infection.

5.7.4.4.3.2.1 Pipelines and hoses. Linked to the previous item is the undoubted fact that it is not at all uncommon for the plant to have excessively long and complicated runs of transfer pipelines with sharp bends and deadspots. This allows the accumulation of materials, water and product often diluted with wash water. Microbiological contamination of the then dilute dispersion can occur rapidly in such pipelines, which can easily become a source of inoculum for fresh product that is pumped through the system. Furthermore, flexible hoses used for transfer of product can, if improperly cleaned and stored, also become contaminated, again especially if diluted product, resulting from ineffective cleaning, accumulates.

5.7.4.4.3.2.2 Mixing vessels and storage tanks. Microbially contaminated vessels and bulk storage tanks, at either the manufacturer or the end user, may be an important source of potential infection.

Open hatches for the passage of air during the filling and emptying of storage tanks may allow aerial inoculation of the product with contaminating microorganisms from the environment. This can easily be resolved by fitting an air filter suitable to permit the passage of air, preventing a vacuum from building up whilst filtering out particulate airborne particulate matter with adhering microorganisms.

Briggs (1980) noted that condensation in the headspace of mixing and storage vessels will wash contaminating microorganisms onto the surface of the bulk phase of the product where, especially if the vessel remains unstirred for some time, dilution of the surface layer occurs along with some degree of syneresis of the product and hence dilution of the biocide, allowing profuse microbial growth to occur. When mixing recommences, a high microbial loading will enter the bulk phase and may 'overwhelm' a biocide, as well as possibly releasing degradative enzymes such as cellulases or amylases (Figure 5).

Empty vessels and storage tanks should be cleaned down at regular intervals but the residual wash water, if left in them, is effectively diluted product and will rapidly become contaminated, even in just a few hours in a

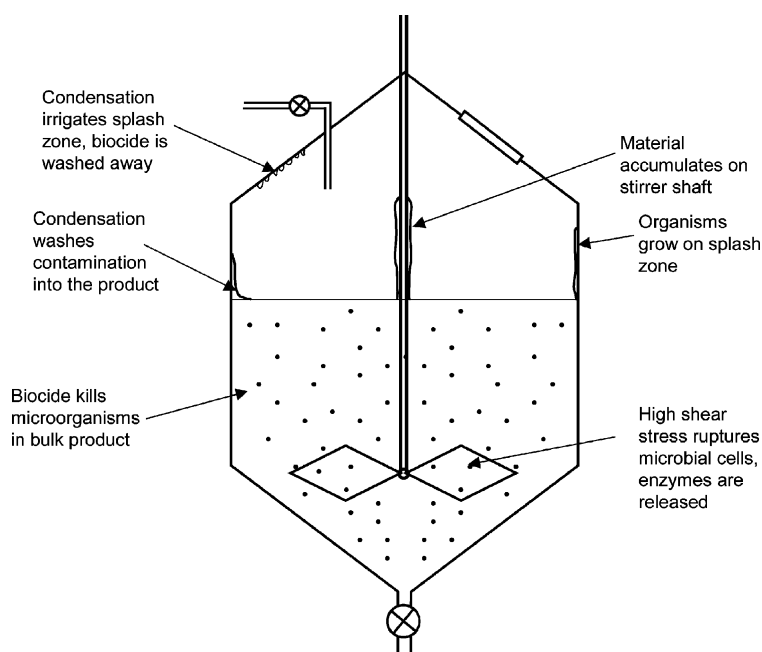


Figure 5 Contamination of a mixing vessel.

warm environment. If the residual wash water is left in the vessel or tank it will provide a heavy source of contamination for the next batch produced. The use of an effective biocide or disinfectant can be employed to prevent this situation from arising.

In addition to regular cleaning and sterilising of vessels and storage tanks, other measures can be undertaken to prevent problems in such equipment. These can include fitting a stirrer to ensure the product is well mixed at the surface and in the bulk or the installation of a simple device to spray a dilute biocide or disinfectant onto the surface of the product at regular intervals. Either of these options will minimise microbial contamination in vessels and storage vessels.

5.7.4.4.3 The transport system. After production the finished polymer dispersion, hopefully in an uncontaminated state, will be either filled directly into containers to be transported to the end user or, more likely, held in a large storage tank and dispensed from there. From the product leaving the manufacturing plant to its application by the end user there are a number of further stages at which contamination may occur.

5.7.4.4.4.1 Drums and containers. Containers used for the transport of polymer dispersions will include small kegs, drums, intermediate bulk containers (one-way or reusable), in-situ or demountable road containers and road and rail tankers. If a sterile emulsion is filled into a sterile, impervious container, microbial growth will not occur but this is very seldom the case and it is often only the biocide that prevents inherent contamination from causing infection.

Firstly the container itself may not be biologically clean. If stored in unsuitable conditions, microorganisms may be present before the product is filled into it. Plastic containers will often attract microbial spores and dusts electrostatically and these can lead to contamination of the product when it is filled into the container. When the container is filled and the lid is applied, condensation may irrigate the lid and form a pool of diluted, under-protected product on the product's surface in which contaminants can grow. It is therefore important that biocide is used that has broad spectrum activity against bacteria, yeast and the mould fungi as well as some degree of headspace protection and is stable in the product for the duration of the product's life.

Reusable intermediate bulk containers (IBCs) are a particular problem. They are often mistreated by the end user and usually inadequately cleaned prior to return for refilling. Dried polymer dispersion deposits are notoriously difficult to remove and it is frequently the case that such containers contain low levels of contamination for the whole of their useful life. Such low concentrations of microorganisms can readily become problematic should the biocide concentration in the dispersion be decreased by dilution or inactivation.

5.7.4.4.4.2 Road and rail tankers. As with IBCs, road and rail tankers are often not cleaned properly between loads and therefore may be an important source of microbial contaminants. The author has experience of a road tanker having been previously used for transporting raspberry jam being then used for polymer dispersion without being adequately cleaned. Therefore, strict guidelines should be rigorously adhered to, ensuring that such containers are properly treated between loads and, as with storage tanks, a biocide should be added to the residual wash water.

5.7.5 Prevention and control of microorganisms in polymer dispersions

The previous sections of this chapter have emphasised the susceptibility of synthetic polymer dispersions, the ease with which they can be spoiled by microorganisms and some of the effects of contamination both in the dispersions themselves and the formulated products in which they may be used. To prevent microbial spoilage and so retain their beneficial properties, it is essential that biodeterioration be prevented. The two ways of doing so are:

- control of the organisms that may come into contact with the product
- the use of a sufficiently effective biocide

It is also very important to ensure that the biocide used is stable in the product for the period over which protection is required. The two main means of preventing infection are equally applicable to the end user of the dispersion, e.g. the paint, adhesive or ink manufacturer to ensure that they too get trouble-free performance from the product.

Biocides are designed to protect uncontaminated products from occasional microbial contamination and, at typical use concentrations, most preservatives are not able to withstand continual repeated challenges of high levels of microorganisms. In such situations, the biocide may be consumed and the product will no longer be protected from subsequent microbial infection.

Very few biocides have a high enough vapour pressure to diffuse into the headspace of mixing vessels, storage tanks and containers, giving some degree of protection to those areas of potential microbial infection foci. Formaldehyde is one of the few preservatives that can fulfil this requirement but, as will be seen later, the use

of this highly effective biocide is declining as a result of regulatory and misunderstood data about its potential toxic effects.

Unlike formaldehyde, most other preservatives have very low vapour pressures, which result in little or no preservative present in the storage tank headspaces. As has already been mentioned, microorganisms introduced into the headspace of a storage tank can often proliferate in the condensation on the walls and ceiling of the tank and on the agitator shaft. When new material is added to the tank, all of the preservative can be consumed in eradicating these microorganisms.

In addition, the greater the range of microorganisms exposed to a preservative system, the greater the likelihood of encountering microorganisms able to survive in the presence of the biocide. Thus, continued poor storage and handling conditions can lead to development of a microbial population which is not controlled by the preservative, especially when inadequate levels are used. In situations in which high microbial populations are exposed to inadequate concentrations of biocide, some microorganisms may respond to the preservative and alter their susceptibility. Development of a resistant, or more precisely tolerant, microbial population is a common problem for some biocides such as formaldehyde. Nevertheless, due to the complex chemical nature of industrial biocides true resistance, in the way that pathogenic species become resistant to antibiotics, is an extremely uncommon occurrence.

5.7.5.1 *Avoiding contamination*

The author previously noted that the use of a biocide alone, however active, is not the complete solution to infection, as many manufacturers have found to their cost (Gillatt, 1993). To mitigate against the effects of microbial contamination it is necessary to adopt an integrated approach to the control of microorganisms, fulfilling the requirements mentioned above. In addition, it needs to be emphasised that whatever steps are followed there will always be some degree of contamination in either raw materials, the plant itself or the products being manufactured. Polymer dispersion manufacture is not the same as production of pharmaceuticals and dispersion manufacturing plants can be very dirty places!

Cresswell and Holland (1995) proposed that microbial contamination in storage tanks could be greatly reduced or eliminated if the following procedures were implemented in conjunction with the use of adequate levels of suitable preservatives:

1. The supply of a microbe-free atmosphere to the tank headspace. After thoroughly cleaning and sanitising the storage tanks, options such as the following can be initiated:
 - a. Use of nitrogen or air purified with ultraviolet radiation or sub-micron filters, or use of clean compressed air.
 - b. Keeping a small positive pressure (a few of centimetres of water) of the clean atmosphere in the tank, or maintaining a low continuous flow through the tank. The advantage of a low continuous flow is a significant reduction in condensation. If using a low continuous flow, care should be taken to ensure that it sweeps the entire headspace and that no other air enters the tank.
2. The microbial quality of raw materials and products, particularly water should be monitored.

As already noted, the microbiological quality of water supplies varies considerably. Boreholes and wells usually contain relatively high levels of a range of organisms including *Pseudomonas* spp. Thus it is important to maintain water quality by the adequate use of chlorine, or other biocides or even by physical agents such as ultraviolet sterilisation systems and more recently by the use of ozone. Deionised water, which is stored, generally needs to be chlorinated to prevent microbial growth. Maintaining a residual chlorine level of 2 ppm in water storage tanks is usually an excellent means of controlling microbial growth.

Heavily contaminated raw materials, especially those that are aqueous-based, e.g. surfactants, defoamers, emulsifiers, thickeners and colloids, should not be used in the manufacturing process and should be replaced. Even the addition of a high concentration of biocide to such materials, whilst possibly killing the infecting microorganisms, will not destroy cellulolytic enzymes, which can retain their activity for a long time after the living cells have died.

Typical microbiological tests for quality control of incoming raw materials, including water and of finished products, can range from agar plate techniques using specific microbiological growth media or dipslides to rapid methods such as impedance measurements and DNA detection.
3. Steps should be taken to ensure that microorganisms are not introduced into the storage tanks when new shipments, batches, etc., are pumped introduced:
 - a. All pipelines and hoses should be flushed and sanitised frequently and product should not be allowed to stagnate in these.
 - b. When possible, bends in piping and hoses should be eliminated and valves, lips, ridges and lines that do not drain (<5% slope) should be carefully and frequently cleaned. These all provide excellent environments for bacteria to adhere to surfaces and establish biofilms.

- c. Care should be taken to avoid the ingress of ambient air into storage tanks when new additions are made.
- d. The introduction of microorganisms into storage tanks by level indicator air sources should be avoided wherever possible. If level indicators bubble ambient air through the tank, consideration should be given to installing a sub-micron filter in the airline or a change to purified compressed air made.

5.7.5.2 Improving plant hygiene

In order to prevent contamination problems, a suitable plant hygiene programme is extremely important (Rigalsford, 1987; Siegert, 1994; Siegert, 1993). Improved plant sanitation is a better and cheaper option than simply increasing preservative concentration to prevent infection, can bring about radical improvements in product quality and reduce biocide usage.

The implementation of a planned plant hygiene programme involving regular audits, assisted by external experts, if necessary, should be a priority. This can be assisted by the implementation of a plant hygiene checklist (Table 12) which can be included as part of in-house quality systems (Gillatt, 1993).

All good dispersion producers and end users will have such a regime in place. Typically, the cleaning procedure and sanitisation programme will involve regular cleaning of storage tanks, pipework etc. with high pressure water jetting apparatus to remove polymer residues on surfaces, combined with caustic washing and application of an appropriate biocide solution. In the author's experience a 1% solution of peracetic acid, liberating hydrogen peroxide, is very effective at removing bacterial biofilm and sterilising surfaces, including the inside of pipework. However, it is very corrosive to metals and great care needs to be taken in its use.

A thorough plant clean down carried out three to four times a year at both dispersion producer's and the end-users' plants will normally be adequate to reduce the risk of microbial contamination. Additionally, this plant hygiene programme should be carried out in the event of an infection prior to refilling with fresh product. To prevent skinning at the dispersions surface as well as surface contamination at the air interface the most appropriate design of bulk tank is one with a stirrer. This greatly improves storage times and quality of the product.

A key component of a plant hygiene programme is worker involvement. It is often the case, especially when production levels are very high, that it is difficult to make time for proper plant cleaning. Awareness by the workforce of the importance and consequences of microbial spoilage is a key factor in maintaining good plant hygiene and avoiding contamination problems. Some manufacturers make greater attempts than others at workforce awareness as shown by Figure 6.

5.7.5.3 The use of biocides

5.7.5.3.1 Required properties of polymer dispersion biocides. It is important that polymer dispersions are adequately preserved and to this end a biocide is added during the manufacturing process (Wood, 1982; Springle, 1990). The type and concentration of biocide used will depend on a number of factors but it is important that the one chosen has the correct necessary properties if the degree of protection required is to be achieved. Generally, the dispersion manufacturers aim to protect and preserve their products against microbial spoilage under reasonably good storage conditions for up to 12 months. It is not the aim to add sufficient preservative to provide protection for any finished product into which it may be incorporated.

Table 12 Plant hygiene checklist

-
- Treat the water supply
 - Add biocide as early as possible during the production process but taking care to maintain its stability
 - Protect stock thickeners and other stock solutions with biocide
 - Avoid surface pooling of condensation water
 - Treat water overlays with biocide
 - Clean down frequently and thoroughly
 - Add biocide to residual wash water
 - Use a biocidal wash
 - Avoid long pipework runs, deadspots, sharp bends
 - Keep flexible hoses clean and dry
 - Pay attention to filling machinery
 - Ensure storage tanks and transport tanks are thoroughly and frequently cleaned
 - Keep empty containers and their lids clean and dry
 - Be aware of problems with plastic containers – electrostatic attraction of dust, mould release agents, etc.
 - Keep the factory as clean as possible
-



Figure 6 Increasing workforce awareness of microbiological problems.

The key essential properties of biocides for use in polymer dispersions were listed by the author (Gillatt, 1990) and are given in Table 13.

Published work (above) and experience has shown that a very wide range of microorganisms can cause contamination of polymer dispersions. Thus a biocide to be used in such products must be able to control all such organisms – bacteria, moulds and yeasts, at a cost effective concentration.

The pH range of polymer dispersions is from 2 to 10, although the majority are in the range 4 to 9.5. Thus a biocide must not only be stable but must be active over that range.

Temperatures as high as 90°C are often reached during manufacture and, although the biocide will often be added at a much lower temperature, the product is often stored in insulated tanks and cooling may take several days. Unreported work by the author looked at the change in temperature of a polymer dispersion filled into a storage tank of 210, 000 litre capacity. Assuming the tank's height and diameter were the same, that the heat transfer coefficient was $\mu = \text{CH}\mu/\text{hr}/\text{m}^2\text{°C}$, that the container was filled in one shot with product at 50°C then the temperature of the dispersion after 10 days at varying mean air temperatures can be calculated as follows:

Mean air temperature	Polymer dispersion temperature after 10 days
25°C	33°C
15°C	27°C
10°C	23°C

Since most storage tanks are insulated, the effect will be even greater in many instances. It should also be remembered that dispersion is often constantly being pumped into the storage tank and removed from it. Therefore the product temperature may often be almost permanently elevated.

Products may contain residual redox agents, for example those listed in Table 14, especially if such materials are used to reduce the residual monomer content of the dispersion. Therefore stability in the presence of these products is an important property of a biocide.

Table 13 Essential properties of polymer dispersion biocides

- Broad-spectrum antimicrobial activity
- Stable over a wide pH range
- Stable at elevated temperatures (to 50°C)
- Stable to various chemical agents
- Compatible with a wide range of polymer types
- Low environmental impact
- Low toxicity and ecotoxicology
- Ease of handling and safe to factory workers
- Relevant regulatory approvals
- Cost effective

Table 14 Some commonly used redox agents

-
- Ammonium, potassium and sodium persulphates
 - Ascorbic acid and ascorbates
 - Bisulphites and metabisulphites
 - t-butyl hydroperoxide
 - Hydrogen peroxide
 - Sodium formaldehyde sulphonylate
 - Sodium nitrite
 - Sodium thiosulphate
-

Table 15 The effect of redox on the stability of isothiazolinones

Redox potential	Stability of CIT/MIT based biocides	Stability of BIT based biocides
> +100 mV	good	unstable
+50 to +100 mV	moderate	very poor
0 to +50 mV	poor	poor
0 to -50 mV	unstable	moderate
< -50 mV	very unstable	good

The author (Gillatt, 1997) commented on work to investigate the relative stability of two of the main biocidal products, the combination of 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CIT/MIT) [II, 15.3.]* and 1,2-benzisothiazolin-3-one (BIT) [II, 15.6.] at various reduction-oxidation (redox) potentials.

A large number of redox potential determinations were carried out on freshly manufactured polymer dispersions using a silver/silver chloride electrode and the results compared with isothiazolin-3-one analyses by HPLC. The conclusions from this work are displayed in Table 15 and confirm the expected finding that CIT/MIT has better stability at positive redox potential values whereas the reverse is generally the case for BIT.

However, great care must be taken if it is intended to put such findings to practical use. It is known that certain redox agents are more aggressive to isothiazolin-3-one biocides than others. For example, Conquer (1993) found that sodium formaldehyde sulphonylate (formasol) destabilised CIT over a wide pH range and persulphates had a similar effect on BIT.

The author also investigated the comparative effects of physical and chemical monomer reduction (stripping) on chloromethyl and methyl isothiazolinones in a polyvinyl acetate/butyl acrylate co-polymer dispersion in a large scale plant trial. Results are detailed in Table 16 and show that even under apparently strongly oxidising conditions CIT was degraded by up to 75% when redox monomer reduction was used. In comparison no such effect was seen with the physically stripped samples.

The results confirm the caution of Cresswell (1996) against the simplistic approach of merely using redox potential measurements to predict isothiazolin-3-one stability. He found that, especially in the case of CIT, the actual concentration of reductant was most important.

He measured redox agent concentrations with time during and after polymerisation and showed that excess oxidant can often be present even when reductant is still active (Figure 7) and described the period between reductant/oxidant crossover as a possible “danger zone” for CIT based biocides.

Only by chemical analysis can the presence of potentially degradative reducing or oxidising agents be determined and their likely effect be taken into account when instituting biocide dosing regimes.

Microorganisms inhabit the aqueous phase of dispersions and, therefore, if the biocide migrates preferentially into the non-aqueous part, its activity may be diminished as it will become unavailable. Although this has been shown to occur it is also the case, with certain such active substances, that an equilibrium is formed between the portion in the two phases. More biocide then migrates into the aqueous part as the biocide concentration there decreases due to its “killing effect”.

Compatibility between biocides and polymer dispersions will vary according to the type, pH and other characteristics of both the biocide and the product in which it is used. Few biocides will be completely compatible with all formulations but such compatibility is important if physical as well as microbiological problems are to be avoided. Many dispersions are sensitive to polyvalent metal ions which can cause coagulation or even emulsion splitting. Therefore there may be problems when biocides stabilised with inorganic Cu^{2+} or Mg^{2+} compounds are used in such products, necessitating the use of a metal salt-free or monovalent stabilised formulation.

*see Part Two – Microbicide Data

Table 16 Effect of physical and chemical monomer reduction on 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) [II, 15.2.] stability

	Physically stripped		Chemically stripped	
	20 ppm CIT	10 ppm CIT	20 ppm CIT	10 ppm CIT
pH after 2 days at ambient	6.5	6.5	6.5	6.5
Redox Potential (mV) after 2 days at ambient	+304	+304	+289	+289
CIT (ppm) after 2 days at ambient	22	12	11	5
CIT (ppm) after 4 weeks @ 40°C	22	11	5	5

Low toxicity/ecotoxicity requirements have led to changes during the last few decades in the types of biocides that can be used. Effective activity in the polymer dispersion allied with low mammalian toxicity and the ability to break down on entering the environment are increasingly important prerequisites for a biocide.

Many polymer dispersions are used in products that may be in direct or indirect contact with foodstuffs, e.g. food packaging adhesives or with the human skin. Thus approval by the relevant regulatory authorities such as the US Food and Drug Administration (FDA) and the German Bundesinstitut für Risikobewertung (BfR) is often essential. Key approval requirements are:

US FDA 21 CFR 175.105	Adhesives
US FDA 21 CFR 176.170	Components of paper and paperboard in contact with aqueous and fatty foods
US FDA 21 CFR 176.180	Components of paper and paperboard in contact with dry food
US FDA 21 CFR 177.2600	Rubber articles intended for repeated use
BfR Recommendation XIV	Preservatives for polymer emulsions in contact with food

Cost effectiveness is another important consideration. Biocides with a low unit cost, for example formalin, have often been used in the past but, on several grounds, including longer lasting effectiveness at low concentrations, biocides with a higher unit cost have been widely adopted in recent years. The important calculation is that the price per kilo multiplied by the concentration required for adequate preservation, must be acceptable to the polymer dispersion manufacturer.

5.7.5.3.2 Biocides for polymer dispersions. Of the large number of biocidal actives available worldwide it is interesting to note that only 330 have are listed in the Notification phase of the EU Biocidal Products Directive (EU, 2002). Of these, less than 100 have been Notified for application in Product Group 6 (in-can biocides) and only a very small number of these are routinely used for the preservation of polymer dispersions.

Biocides used for the protection of polymer dispersions have changed, as the user industry has demanded improved properties. For example, heavy metal based biocides such as those containing mercury have now

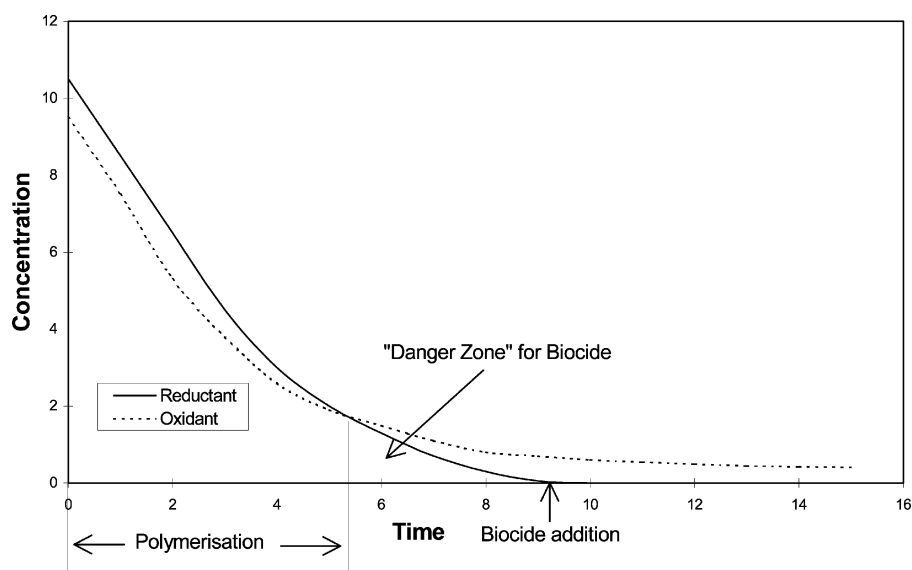
**Figure 7** Redox chemical concentration vs time during dispersion polymerisation.

Table 17 Biocides used in polymer dispersions

-
- Heavy metals, e.g. Mercury based [II, 19.]
 - Formaldehyde/formalin [II, 2.1.]
 - Formaldehyde donors, e.g. Triazines, adamantane derivatives [II, 3.]
 - Compounds with activated halogen atoms, e.g. 1,2-dibromo-2,4-dicyanobutane (DBDCB) [II, 17.16.] and 2-bromo-2-nitropropane-1,3-diol (Bronopol) [II, 17.14.]
 - 1,2 benzisothiazolin-3-one (BIT) [II, 15.6.]
 - Combination of:-
 - 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and
 - 2-methyl-4-isothiazolin-3-one (MIT) [III, 15.3.]
 - 2-methyl-4-isothiazolin-3-one [II, 15.1]
 - Combination of:-
 - 2-methyl-4-isothiazolin-3-one and
 - 1,2 benzisothiazolin-3-one
-

disappeared from use and whilst formaldehyde and formaldehyde donors are still used for reasons of low cost, these products are also less frequently encountered.

Some of the main active substances that have been that have been incorporated into biocide formulations in at past and present were listed by the author (Gillatt, 1993) and are summarised in Table 17.

The recent use of high concentrations MIT and the widespread use of the synergistic blend of MIT and BIT are in response to the EU 15 ppm labelling limit for CIT/MIT combination biocides.

5.7.5.3.2.1 Heavy metal biocides [II, 19.]. Paulus (1993) commented on the decline in the use of heavy metal biocides in general and mercury based biocides in particular. He said that there is a strong movement throughout the world in favour of their substitution in view of their toxicity and especially their ecotoxicity.

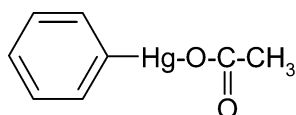
Organomercury compounds, in particular, have already been largely substituted and are no longer of much importance as microbicides for material protection. In the truest sense of the word they may be termed biocides, since they are effective not only against microbes, but also against all forms of life.

Although Hippocrates already knew of the toxicity of mercury compounds he could not anticipate the environmental problems that were to arise through their excessive use - problems rooted in the fact that these compounds are biodegradable only to the stage of methyl- or dimethylmercury, both of which are extremely toxic (Lakowitz & Anderson, 1980). They diffuse rapidly across permeability layers such as membranes. Methyl- and dimethylmercury therefore accumulate in living organisms, especially those at the end of the food chain, such as predatory fish and animals dependent on fish. Without this accumulation the lethal doses of mercury compounds would be less problematical. If a quantity of mercury compounds is absorbed by a human, about 10% of it is carried to the brain. Long-term exposure to mercury compounds is particularly dangerous, even accumulation at very low rates causing manifest damage to the brain and nervous system after a number of years.

Mercury compounds, even at extremely low concentrations, are environmentally unsafe for another reason: investigations have shown that, at concentrations as low as 1 ppb, mercury-based microbicides cause 50% inhibition of the photosynthesis of phytoplankton and that at 50 ppb this photosynthesis stops entirely (Harries et al., 1970). Concentrations of this order are also toxic to fish.

5.7.5.3.2.2 Formaldehyde [II, 1] and formaldehyde donors [II, 3.]. Aldehydes [II, 2.] in general are another group of biocidal actives, widely used in the past but less frequently encountered today. Mention has already been made about the advantageous property of the volatility of formaldehyde giving so-called “headspace protection” to closed containers. In addition formaldehyde has a rapid rate of kill and is extremely inexpensive.

However, due to its volatility the effect of formaldehyde can be short lived, especially when used in warm products or in those stored at elevated temperature. In addition it will lose its activity in the presence of some organic materials and has more recently been shown to cause coagulation of certain new types of polymer dispersion.

**Figure 8** Phenylmercuric acetate.

The biocidal efficacy of formaldehyde is predominantly against bacteria with concentrations appreciably above the R40 (*"danger of irreversible effects"*) level of 1,000 ppm being required to control some yeasts, moulds and tolerant bacteria.

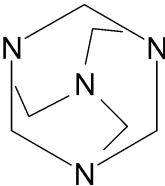
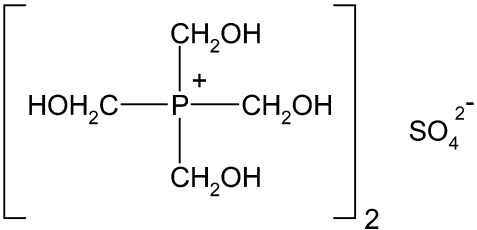
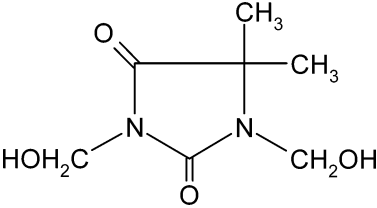
Nevertheless because of its low cost, rapid efficacy and headspace effects formaldehyde is still a popular biocide, especially when combined with more modern actives such as chloromethyl and methyl isothiazolinones.

Formaldehyde may be used as formalin or paraformaldehyde or, more commonly as a formaldehyde condensation product able to release the active substance once present in the product it is designed to protect.

Paulus (1993) described a large number of different classes of formaldehyde donor, including examples given in Table 18. By definition, the microbiological efficacy of formaldehyde donors is explained by their release of formaldehyde. Thus, the biocidal effect is achieved by the free formaldehyde and not by the donor itself. In aqueous medium, the formaldehyde donor is at equilibrium with free formaldehyde, i.e. in aqueous solution there is always a certain amounts of formaldehyde present in a free form. Formaldehyde reacts with essential, nucleophilic parts of the microbial cell. The formaldehyde, which is thereby consumed, will be replaced by hydrolytic separation from the donor. This is especially the case in the periphery of the cytoplasmic membrane of aerobic cells, which is rich of protons and where there is often a release of formaldehyde from donors.

Other commonly encountered formaldehyde donors include hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine [II,3.3.18], a monoethanolamine formaldehyde condensate. It is cheap and compatible with many products, although it is known to cause discolouration in some. In addition it has poor thermal stability and has recently been placed on Annex 1 of the Dangerous Substances Directive having been identified as a potential skin sensitiser. As a result, formulations containing efficacious levels of this class of triazine are required to be labelled

Table 18 Some formaldehyde donor biocides

Hemi-formals[II, 3.1.]	$\text{CH}_3\text{-(CH}_2\text{)}_2\text{-CH}_2\text{-O-CH}_2\text{-OH}$ Butanolhemiformal [II, 3.1.1.]
Amine/formaldehyde reaction products [II, 3.3.]	 Hexamethylenetetramine [II, 3.3.1.]
Amide/formaldehyde reaction products [II, 3.3.]	$\text{Cl-CH}_2\text{-CO-NH-CH}_2\text{OH}$ N-methylolchloracetamide [II, 3.4.1.]
Quaternary phosphonium salts	 Tetra-(hydroxymethyl)-phosphonium sulphate (THPS) [II, 3.6.]
Hydantoins	 1,3-bis(hydroxymethyl)-5,5-dimethyl-2,4-dioxo-imidazolidine (DMDM Hydantoin) [II, 3.4.9.]

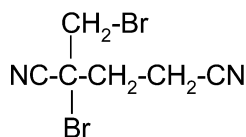


Figure 9 DBDCB.

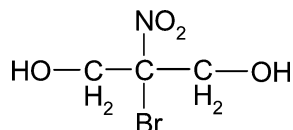


Figure 10 Bronopol.

R43 (“may cause sensitisation by skin contact”). for these and other reasons the triazine formaldehyde releasers and the oxazolidines, e.g. bis-(5,5'-dimethyl-1,3-oxazolidin-3-yl)-methane are rarely found protecting polymer dispersions.

5.7.5.3.2.3 Compounds with activated halogen atoms[II, 17.].

5.7.5.3.2.3.1 *The cyanobutanes.* The main example of this product type is 1,2-dibromo-2,4-dicyanobutane [II, 17.18] (DBDCB – Figure 9). It is reasonably effective, except at alkaline conditions. Paulus (1993) said that, according to its chemical structure, it contains several highly withdrawing centres making the compound strongly reactive with nucleophilic groups in the microbial cell. As a result it has broad spectrum biocidal efficacy but is known to cause discolouration in some products and has poor water solubility.

Because DBDCB is approved for use in cosmetics it is perceived as a “safe” product and is used in the polymer dispersion industry in products destined for the production of non-woven fabrics which may have intimate human body contact, e.g. disposable nappies and feminine hygiene products. However, in comparison with other polymer dispersion biocides it is an expensive product and its use is restricted to niche applications.

5.7.5.3.2.3.2 *2-Bromo-2-nitropropane-1,3-diol (Bronopol – Figure 10)* [II, 17.14] was previously classified as a formaldehyde donor (Paulus, 1993) as it is possible to demonstrate release of up to 30% of its theoretical content in dilute solution at alkaline pH. However, the amount of formaldehyde theoretically able to be released from Bronopol is far too low to account for its efficacy, e.g. 25 ppm from 200 ppm of Bronopol, when levels of formaldehyde in the range 500 – 1,000 ppm are necessary. Therefore Scheid (2002) believed that any formaldehyde evolved plays little or no part in its biocidal activity.

Bronopol is rarely used alone for the preservation of polymer dispersions, being most commonly part of a combination biocide system, e.g. with chloromethyl/methyl isothiazolinone. In such formulations it helps to eliminate the weakness of 5-chloro-2-methyl-4-isothiazolin-3-one against anaerobic sulphate reducing bacteria and has been shown to be effective against slow growing acidophilic species such as *Gluconoacetobacter liquefaciens* (Herrick, 2001).

Table 19 Isothiazolinone biocides for the preservation of polymer dispersions

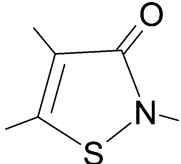
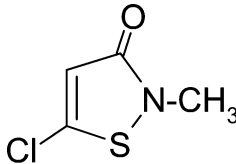
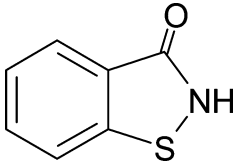
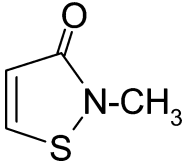
 The isothiazolin-3-one ring	 5-chloro-2-methyl-4-isothiazolin-3-one
 1,2-benzisothiazolin-3-one	 2-methyl-4-isothiazolin-3-one

Table 20 A comparison of CIT/MIT and BIT

Property	CIT/MIT	BIT
pH as supplied	acidic	alkaline (some > pH 12)
pH stability	2–9	2–12
Water solubility	complete	poor (100 ppm)
Temperature stability	< 65°C	100°C
Best biocidal activity	pH 2–9	> pH 8
1°, 2° amines	unstable	stable
Oxidising agents	stable	unstable
Reducing agents	unstable	stable
Sulphydryl groups	unstable	stable
Biocidal activity:		
Bacteria	very broad	restricted (poor activity against <i>Pseudomonas</i> spp.)
Moulds	very broad	poor
Yeasts	very broad	poor
Speed of kill	rapid	slow

5.7.5.3.2.4 *The isothiazolinones [II.15.]*. By far the most widely used group of polymer dispersion biocides are those based on the isothiazolin-3-one structure and this and the principle active substances of this class are depicted in Table 19

First used almost 30 years ago, 1,2-benzisothiazolin-3-one (BIT) rapidly became the benchmark biocide for the preservation of polymer dispersions and was the single most widely used active substance until the introduction of the blend of 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) and 2-methyl-4-isothiazolin-3-one (MIT), in the approximate ratio 3 : 1, in the early 1980s.

When redox compounds are used, either as initiators or as a means of reducing remaining monomer, the natural tendency is for a small amount of residual reducing agent to be present. It was eventually realised that to prevent the destabilisation of BIT such a net reducing state needs to be present in protected products. In dispersions where the residual redox agent was an oxidiser, especially persulphate, rapid and, on several occasions, catastrophic degradation of BIT occurred.

Polymer dispersion users were initially attracted to the CIT/MIT combination product because of its greater efficacy (often 15 ppm of total CIT/MIT will be effective, compared with 100 ppm or more of BIT) and lower cost. However, polymer dispersions designed, either intentionally or by accident, to maximise the stability of BIT, i.e. net reducing, were found to as rapidly degrade CIT as with oxidising agents and BIT. Once this problem was recognised and resolved CIT/MIT either alone or in combination with other active substances, such as formaldehyde/formaldehyde donors and Bronopol quickly displaced BIT as the polymer dispersion “biocide of choice”

In comparing these two main types of biocide it is important to recognise their unique and different properties (Table 20)

Comparative MIC (Minimum Inhibitory Concentration) data is often misleading unless products are tested against the same organisms at the same time using the same method. Nevertheless there are undeniable differences between the two isothiazolinone types, as shown by Paulus (1993) (Table 21)

Elsom (1988) recognised the combination of chlorinated and non-chlorinated isothiazolinones as being the most cost effective biocides for the protection of polymer dispersions and this is undeniably the case in products in which the halogenated species is stable.

However, the requirement, under the EU’s 28th Adaptation to Technical Progress - ATP (EU, 2001) that products containing CIT/MIT at greater than 15 ppm must display the Xi irritant symbol and the R43 risk

Table 21 Comparative minimum inhibitory concentrations of CIT/MIT and BIT

	MIC (ppm)	
	CIT/MIT	BIT
<i>Corynebacterium</i> sp.	2.5	25
<i>Escherichia coli</i>	2.5	25
<i>Klebsiella</i> sp.	2.5	25
<i>Proteus penneri</i>	2.5	20
<i>Pseudomonas aeruginosa</i>	2.5	150
<i>Pseudomonas putida</i>	2.5	60
<i>Pseudomonas stutzeri</i>	1.0	20
<i>Aspergillus niger</i>	5.0	100
<i>Penicillium funiculosum</i>	1.0	40
<i>Saccharomyces cerevisiae</i>	5.0	15

Table 22 Minimum inhibitory concentrations of 2-methyl-4-isothiazolin-3-one

Organism	MIC (ppm)		
	MIT	BIT	MIT/BIT (1:1)
<i>Escherichia coli</i>	17.5	25	10
<i>Klebsiella pneumoniae</i>	20	25	15
<i>Proteus vulgaris</i>	25	20	10
<i>Pseudomonas aeruginosa</i>	30	150	20
<i>Pseudomonas putida</i>	12.5	60	10
<i>Pseudomonas stutzeri</i>	12.5	20	10
<i>Aspergillus niger</i>	750	100	50
<i>Paecilomyces variotii</i>	100	40	20
<i>Penicillium funiculosum</i>	200	40	20
<i>Saccharomyces cerevisiae</i>	150	15	10

phrase (“*may cause sensitisation by skin contact*”) has lead to a complete re-evaluation of the biocides used in polymer dispersions. Pressure from the end users of polymer dispersions, especially the paint and adhesive industries has resulted in many European manufacturers replacing biocides containing CIT with other actives.

The revaluation of polymer emulsion actives resulting from the 28th ATP labelling limit has included further investigation of the activity of 2-methyl-4-isothiazolin-3-one (MIT), the minor component of the CIT/MIT blend.

MIC data shows MIT to have similar biocidal properties to BIT (Table 22) with both being effective against the majority of species tested (with the exception of *A. niger*) at between 10 and 200 ppm.

However, MIT is stable in the presence of mild reducing as well oxidising agents and has much better pH and thermal stability than CIT. Problematically, high concentrations of MIT are required in practice and at the time of writing it is a very expensive alternative to traditional CIT/MIT-containing formulations.

Because of the cost and timescale for the development of new active substances and the registration and regulatory requirements, there have been no unique biocidal actives widely used for protection of polymer dispersions during the last 20 years, since the chloromethyl/methyl isothiazolinone blend was introduced.

As a result, biocide manufacturers have striven to optimise the efficacy of existing active substances by producing blends with improved properties. Already mentioned have been combinations of CIT/MIT with either formaldehyde/formaldehyde donors or Bronopol which have gained very wide acceptance.

More lately the synergistic blend of 1,2-benzisothiazolin-3-one and 2-methyl-4-isothiazoline-3-one (MIT/BIT) has been recognised as an extremely effective combination for the preservation of polymer dispersions and other formulations. If the MIC data for a 1:1 mixture of MIT and BIT is compared with that of the individual components (Table 21) it can be seen that the total concentration of MIT and BIT required to prevent the growth of each organism is appreciably lower than that of either active substance alone. Such synergy is confirmed when the Synergistic Index of MIT/BIT against one of the most common polymer dispersion infecting bacteria, *Pseudomonas aeruginosa*, is considered (Figure 11).

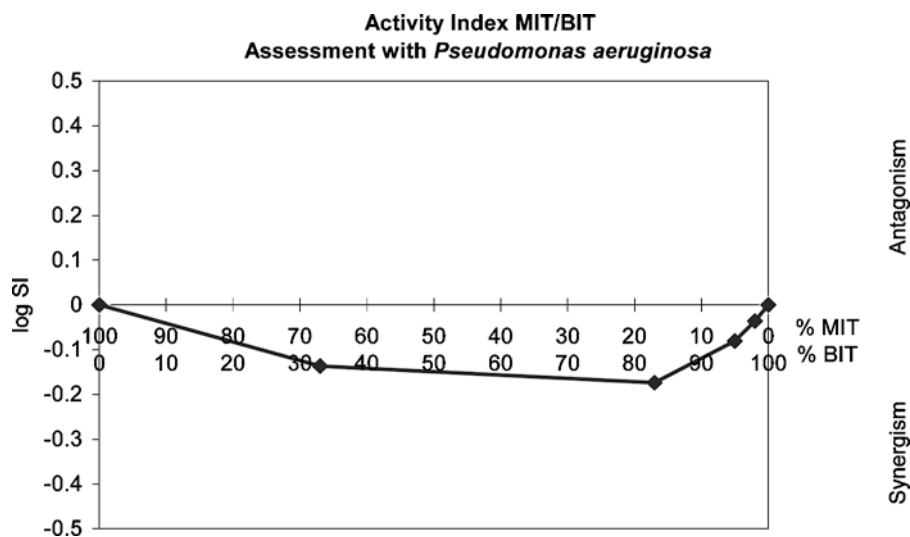
**Figure 11** Synergistic index study of MIT and BIT.

Table 23 Advantages of 2-methyl-4-isothiazolin-3-one/1,2-benzisothiazolin-3-one combination

- AOX (Absorbable Organic Halogen) free
- Solvent and VOC (Volatile Organic Compound) free
- Formaldehyde/aldehyde free
- pH stable at > 9
- Active substances EU Food Contact Additive approved
- US EPA and German BfR approved
- Temperature stable compared with CIT/MIT
- Reductant stable
- Lower skin sensitising potential
- Unaffected by EU labelling restrictions on BIT and CIT/MIT at normal use concentrations
- Broader activity spectrum than BIT

The key properties of the combination of MIT and BIT result in it being regarded as the wet-state biocide for the future, especially in markets where regulatory pressures mean that there are restrictions on CIT/MIT based products (Table 23).

In a Magnusson and Kligman OECD 406 (Magnusson and Kligman, 1969) skin sensitisation study, elicitation concentrations for a 1.5% CIT/MIT biocide were 0.25% whereas the effect level for a 5% total MIT/BIT combination biocide was 5%. The MIT/BIT based product was therefore potentially 20 times less sensitising.

5.7.5.4 Evaluation of polymer dispersion biocides

Prior to adoption, polymer dispersion biocides must be tested in the products they are intended to preserve under conditions as close as possible to those that will be experienced in use.

One of the difficulties inherent in the evaluation of these products is the changing nature of polymer dispersions after manufacture. Often the reaction between added redox chemicals and residual monomer will continue for some time after the dispersion has been produced.

Therefore, in laboratory testing, it is extremely important that the addition of biocide to the polymer dispersion copies as far as possible the conditions that would occur at the production plant. In almost every case a polymer dispersion sample that has been stored prior to addition of biocide will show less degradation of the biocide than if the biocide was added when the polymer dispersion was freshly made. This requires all additions of biocide samples to unprotected polymer dispersions to be made at the polymer dispersion plant or laboratory where the polymer dispersion is produced. As a consequence the additions must be made while the polymer dispersion is at the same temperature, pH and redox state that would occur during biocide addition to a production batch.

If this is not followed, the results of laboratory trials may be misleading and will usually indicate a greater stability of the biocide than will be found in practice (Figure 12). For this reason, blank (biocide free) samples of polymer dispersion should not be used by test laboratories for biocide additions (Roden, 2002).

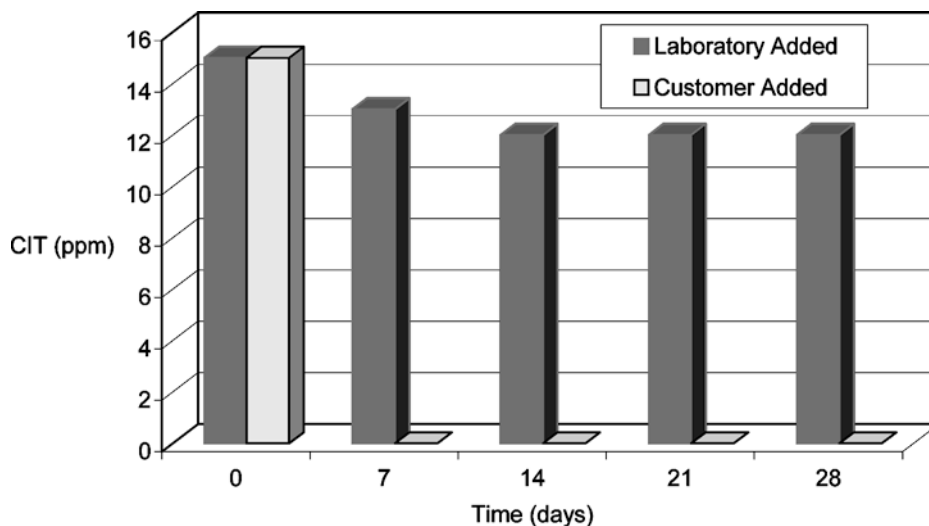
**Figure 12** HPLC analysis of polymer dispersion comparing laboratory and customer added biocide.

Table 24 IBRG Polymer dispersion inoculum

Bacteria		
<i>Alcaligenes faecalis</i>	IMI 358536	DSM 13644
<i>Escherichia coli</i>	IMI 362054	DSM 13631
<i>Proteus vulgaris</i>	IMI 358534	DSM 13625
<i>Pseudomonas aeruginosa</i>	IMI 358539	DSM 13626
<i>Pseudomonas putida</i>	IMI 358533	DSM 13624
<i>Pseudomonas stutzeri</i>	NCIMB 11359	DSM 13627
Yeasts		
<i>Rhodotorula rubra</i>	IMI 358541	DSM 13621
<i>Saccharomyces cerevisiae</i>	IMI 358542	DSM 13622
Filamentous Fungi		
<i>Aspergillus terreus</i>	IMI 358546	DSM 13630
<i>Fusarium solani</i>	IMI 286367	DSM 13628
<i>Geotrichum candidum</i>	IMI 358544	DSM 13629

A number of methods are available for evaluating the microbial resistance of polymer dispersions and biocides added to them but there are, as yet, no recognised international standards. Nevertheless, the work of the International Biodeterioration Research Group (IBRG) in this field has lead to the production of a draft method (IBRG, 2001), which, it is believed, can be used as an efficacy test for biocides within the scope of the European Biocidal Products Directive and for predicting the in-use performance of polymer dispersion biocides.

The IBRG test involves sequential challenge of aliquots of a polymer dispersion with a standardised inoculum (Gillatt, 1995) followed by monitoring of growing or surviving organisms. The IBRG inoculum, developed as part of a collaborative study, comprises the organisms listed in Table 24.

Other organisms known to be appropriate to the specific product under test may be used in addition to or in place of those listed above.

Samples may be aged, e.g. by heating at 40°C for up to four weeks, before challenge to simulate long term storage at elevated temperature, the recovery monitoring intervals between challenges can be altered to determine a biocide's "rate of kill" and more than the specified four challenges can be carried out to ascertain the preservative's "longevity".

5.7.6 Conclusions

There have been many changes in the polymer dispersion industry during the last ten years, mostly aimed at satisfying the needs of the regulators and registration authorities. The growing awareness of the hazards associated with some of the raw materials used in polymer dispersion manufacture, e.g. monomer, has resulted in the industry making strenuous efforts to address these issues and changes such as monomer reduction programmes have been successfully introduced.

Similarly the process of registration and regulation, guided in Europe by the introduction of the Biocidal Products Directive and EU Dangerous Substances and Dangerous Preparations Directives is bringing about marked changes in the biocides used for the preservation of polymer dispersions. Further pressure has been brought about by the polymer dispersion end users, especially in the paints and adhesives industries. From heavy metal and phenolic biocides to BIT, then to CIT/MIT and combination biocides the trend has moved in the direction of CIT-free and AOX/VOC-free preservatives with the combination of 2-methyl-4-isothiazolin-3-one and 1,2-benzisothiazolin-3-one meeting all the required properties and proving extremely effective.

However, given the required properties of polymer dispersion biocides it is unlikely that there will ever be a true "universal preservative". Development costs and regulatory and registration restrictions will prevent the development of novel molecules for polymer dispersion preservation and producers will continue to concentrate on the formulation of a number of active agents that together may fulfil the requirements of the ideal biocide.

5.7.7 Acknowledgements

The author acknowledges the work of K. Holland, formerly of Vinamul Ltd., on the chemistry of polymer dispersions which, in an amended version, forms the major part of the section of this chapter on that topic. The chapter "The Preservation of Aqueous-based Synthetic Polymer Dispersions and Adhesive Formulations" by M.A. Cresswell and K. Holland from *Preservation of Surfactant Formulations*, ed. F.F. Morpeth, Chapman and Hall, 1995 pp. 213–261 has been extensively quoted.

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5.8 Application of microbicides for the storage protection of mineral dispersions

P. SCHWARZENTRUBER and P.A.C. GANE

Introduction

The subject 'Application of Microbicides for the Storage Protection of Mineral Dispersions' is of ever-increasing interest for scientists and industrialists and includes many challenges for the mineral slurry producer and user. Increasing conversion from dry pigment handling to water-based dispersions is taking place over a wide range of production applications, for example, papermaking filler products and coatings formulations in both the paper and paint industries.

The requirements for the delivery of preserved slurried products begins from the moment the mineral is extracted or synthetically produced. The process conditions are as important regarding bacterial colonisation and control as the delivery and storage strategy of the end-product itself. This article attempts to give a brief insight into the background issues and procedures needed to provide an environment of "good housekeeping", essential in optimising the microbiological control needed for preservation and acceptable application of the pigment in its end-use. On this base, the latest research on the bacterial strains, their identification, measurement and colony growth dynamic is presented, and the biocide strategies, applicability and constraints are discussed. Illustrations are given throughout of the sources of microbiological contamination likely to occur during production, storage and transportation.

Based on the current knowledge being gained from combining active Research and Development and on the ground Applications expertise, new possibilities for optimising microbiological quality control are described.

5.8.1 Pigment manufacturing process

From the mining of raw stone to the delivery of a pigment suspension to the paper industry, the material passes through a wide range of processing steps and procedures, typically as might be seen schematically in Figure 1. Following the breaking of the mineral or mineral-containing stone, the first crushing step, the material is usually washed and prepared for optical and size selective sorting. To separate unwanted finely intra- and inter-grown minerals, beneficiation by flotation is often carried out prior to further more stringent size classification by hydrocyclone or centrifuge. If synthetic pigments are to be made, the raw material, such as limestone, prior to burning or calcining, must also be carefully selected. The slaking process for precipitated calcium carbonate, for example, requires specific control of burnt lime sourcing and particle size before the carbon dioxide addition stage.

Adjusting the parameters of wet (waterborne) fillers or pigments to the respective requirements of paper making and coating, is achieved by control of crystal growth, in the case of precipitated products such as precipitated calcium carbonate or precipitated silica, control of particle delamination and comminution, in the cases of kaolin, mica and talc, or by grinding and selection technology in a wet milling process, for materials such as ground calcium carbonate. Each methodology is pursued at a controlled solids content in either a dispersed or a flocculated state, depending on the mineral base and the use of various dispersants and flocculants. Final product dispersions depend in respect to concentration on the chosen particle size and shape distributions, their state of dispersion and the intrinsic particle-particle packing characteristics. When transportation over long distances is required, methods to achieve the maximum solids content within the constraints of the final application are strenuously sought.

During fine grinding processes, for example, temperatures of up to 110°C can be reached. This ensures to a large extent the thermal disinfection of the processed mineral. Thus, there occurs generally a significant reduction in the bacterial count, arising initially from contaminated plant and treatment waters and process additives, from that found in the feed material. After such a grinding or high temperature processing step, the dispersions can often be regarded in microbiological terms as of pharmaceutical quality. There are, however, some cells which, due to a certain protection mechanism, are not destroyed by the grinding process but are transferred into a physiologically stressed or dormant state. During slurry product storage, post-dispersion of synthetic products, or during transportation, the temperature can decrease (or rise again) to a level which is favourable to bacterial growth (25–45°C). This growth then proceeds once either infection occurs from outside sources, usually by fluid contamination or even contact with air, or by the further viability of the dormant or stressed cells. It is this post-processing contamination that focuses most attention when considering slurry preservation. Of course, should a processing step involve lower temperature handling or combinations of products then a complete revision of the process environment in respect to bacterial contamination must be made. This is specific to the plant in question

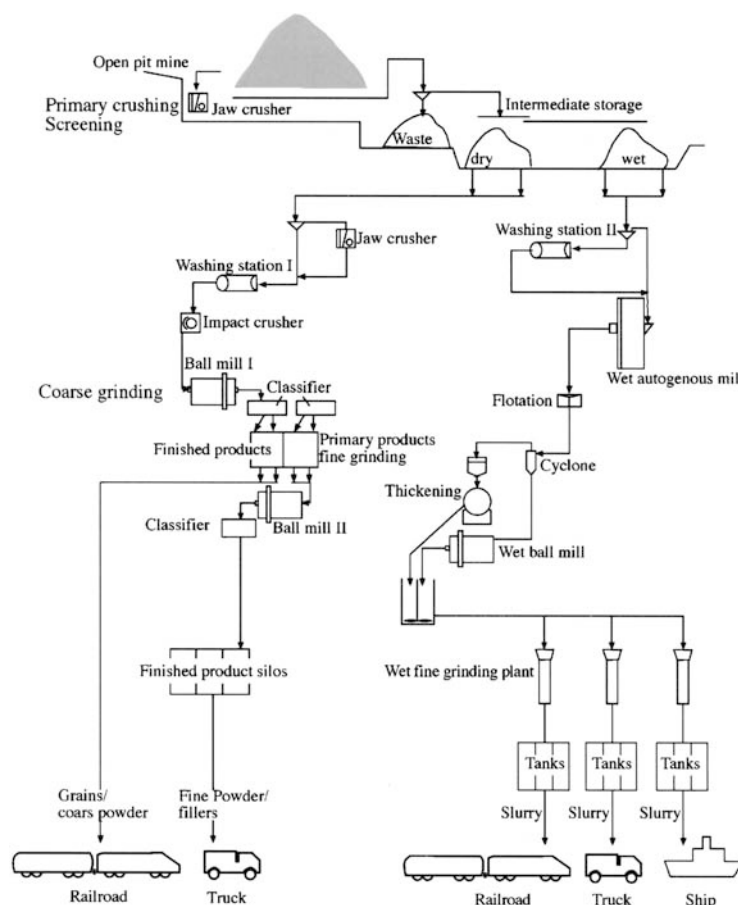


Figure 1 Scheme of a pigment manufacturing process.

and requires extensive analysis of water systems, settling and waste recovery vessels, and air-borne sources, such as air-conditioning, ventilation etc.

5.8.2 Organic additives for dispersion stabilisation – a rich nutrient basis

To be able to produce a suspension having a controlled solids content, often of more than 70 w/w%, special dispersants are required. Without such dispersants, a mixture containing a mineral, such as calcium carbonate, with only 30 w/w% water is no longer flowable. Pigment and filler producers have developed highly active polymer systems, especially for the use of mineral slurries in the paper industry, which allow such concentrations to be achieved without destroying the complex chemistry of a paper machine.

The dispersants, usually based today on salts of polyacrylic acid (PAA), represent a rich supply of organic nutrients for cells. They serve also as both a carbon and energy source. Mineral dispersions, however, also

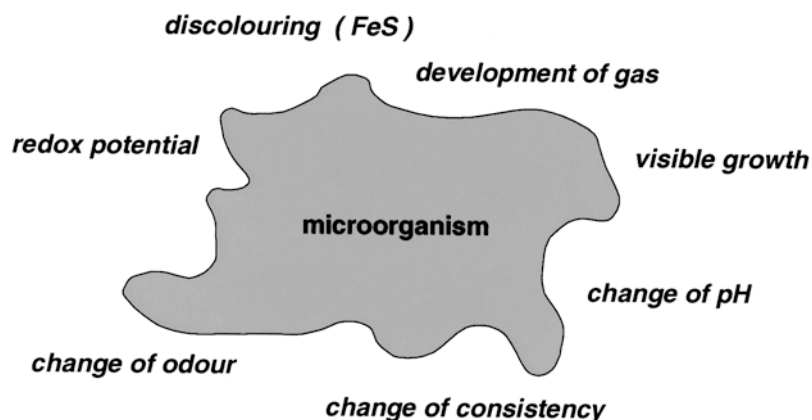


Figure 2 Microbial contamination and its consequences.

contain a series of other important biologically supportive substrates, which contain oxygen, nitrogen, calcium, magnesium, sodium, potassium, phosphorus, sulfur and iron, all of which are essential for energy metabolism.

The microbiological colonies, as well as their growth in a mineral dispersion, can be influenced by many different factors, such as:

- Higher solids contents of a dispersion. High solids content makes growth beyond a certain concentration more difficult as the physical space for microorganisms is reduced. As a result, dispersions with high solids contents are often easier to preserve.

Higher salt concentrations lead to a differential osmotic pressure across the microbial cell walls. The effects are selective to the species present in a system. Usually it is a change in salt concentrations, rather than an absolute level, that can have an inhibiting effect on the growth of microorganisms already extant in the system.

5.8.3 Microbial contamination and its consequences

Microorganisms are omnipresent on earth (and maybe beyond) and, of course, mineral dispersions are no exception. Bacterial counts of $> 10^6$ cfu/ml can lead to unpleasant odour, discoloration, acidification and viscous build-up. Under certain conditions of aeration, followed by stagnation, strong initial aerobic growth, eventually consuming the oxygen present, can subsequently create the conditions for anaerobic growth, which is generally connected with a decrease in the redox potential. Furthermore, the decrease in pH, and the often associated increase in viscosity, can lead to considerable problems for the final user. The rheological properties of mineral dispersions are extremely important for the processing of the product (e.g. pumpability, filtering, rheological flow characteristics in a coating head and in recirculation systems).

Furthermore, there is a risk of uncontrolled deposition (biofilms), which, for example in paper production, could lead to holes and breaks in the paper web.

Clearly, the need for biocide(s) to initiate, preserve and maintain slurry purity is a very important part of the slurry producer's and handler's requirements for efficient application and storage of minerals.

Biocide in the slurry itself, however, is only one aspect of preservation. In order to keep mineral slurries clean in storage tanks and during transportation (truck, railcar, boat) (Photo 1), and to achieve good performance of biocide(s) within the product, the effect of headspace preservation as well as the cleaning of pipes and transportation facilities must not be underestimated (Photo 2). Contamination from headspace can be a major source of biofilm development which has a high potential for the recontamination of mineral slurries (Figures 3 and 4). Biofilms can also occur in places where mechanical cleaning is difficult, i.e. dead corners in pipework and storage or reaction vessels. In these cases, where biofilms flourish there is often a limit to the practical chemical disinfection and preservation that can be attained, due to the impermeability of many of these types of films. The need to avoid stagnation in plant design is, therefore, paramount. See also Chapter 5.1.

The target, therefore, is not only microbial control within the product itself but also the control within the confines of the immediate product-contacting environment.

To meet today's logistic requirements, slurry tank vessels with a capacity of up to 16,000 registered tonnes are in operation.

Photo 1: The transport of these large quantities of mineral slurry dispersion makes great demands upon the preservation and brings a new meaning to being "ship-shape".

Photo 2: Emptied rail tank waggons are cleaned with fresh water under high pressure to guarantee optimum conditions with respect to cleanliness for the next load to be transported.



Photo 1 Transport of large quantities of mineral slurry dispersions.

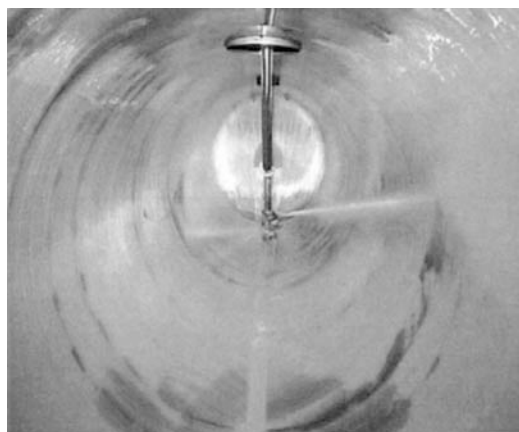


Photo 2 Good housekeeping–Cleaning of transport facilities.

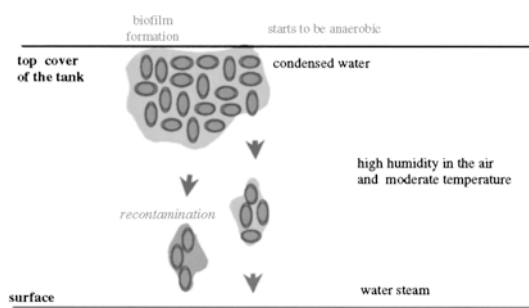


Figure 3 Recontamination by biofilm formation at a top cover of a tank.

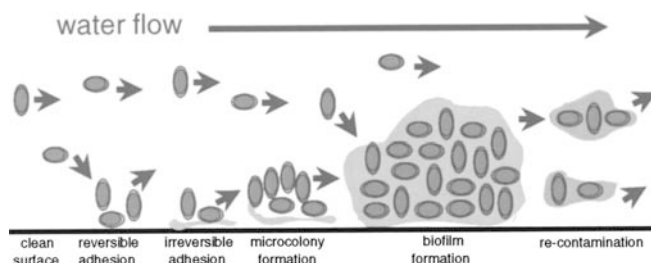


Figure 4 Recontamination by biofilm formation in a pipe.

5.8.4 Diversity of bacterial morphologies

The rich nutrient supply, moderate temperatures during transportation and storage of mineral slurry dispersions, as well as a neutral to slightly alkaline pH (7–10), provide an environmental spectrum of conditions for a large number of microorganisms to develop and thrive.

When investigating the microbial diversity that exists in mineral slurries a number of factors have to be considered. These mostly surround questions of how to perform, and what are the effects of extraction and isolation of the bacteria concerned. For example, it has been demonstrated that standard culture-based techniques only isolate as little as ~1% of the total microbial population found in common soils (Bornemann and Triplett, 1997). Such standard techniques applied to pigment slurries often present similar limitations. Recently, the current authors have shown by microscopic analysis of bacteria concentrated from typical mineral slurries that a diverse range of cell morphologies can be revealed, which by culture techniques remain unidentified (Photo 3 (Schwarzentruher, P., 2001)). Subsequently, the employment of the most modern techniques arising from the field of molecular biology, such as PCR (Polymerase Chain Reaction) amplification of the 16s rDNA, cloning and sequencing and/or RISA (rDNA Internal Spacer Analysis) (Figure 5 and Photo 4 (Schwarzentruher, P., 2001)), enable the identification of some of the major microorganisms found to occur in mineral dispersions.

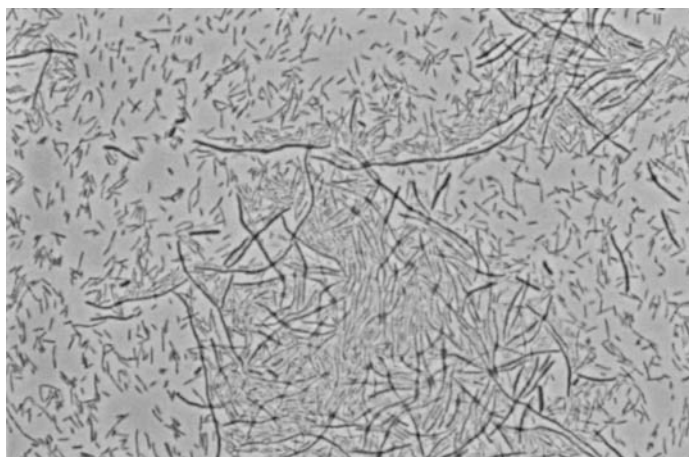


Photo 3 Bacterial diversity in a CaCO_3 slurry sample.

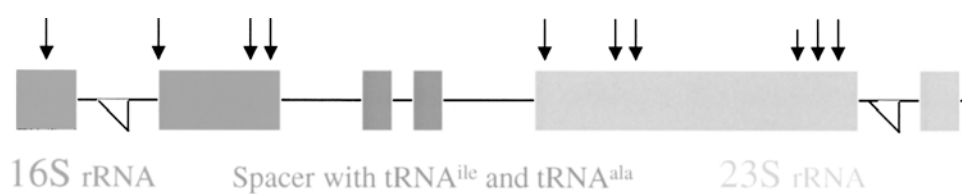


Figure 5 RISA (rDNA Internal Spacer Analysis).

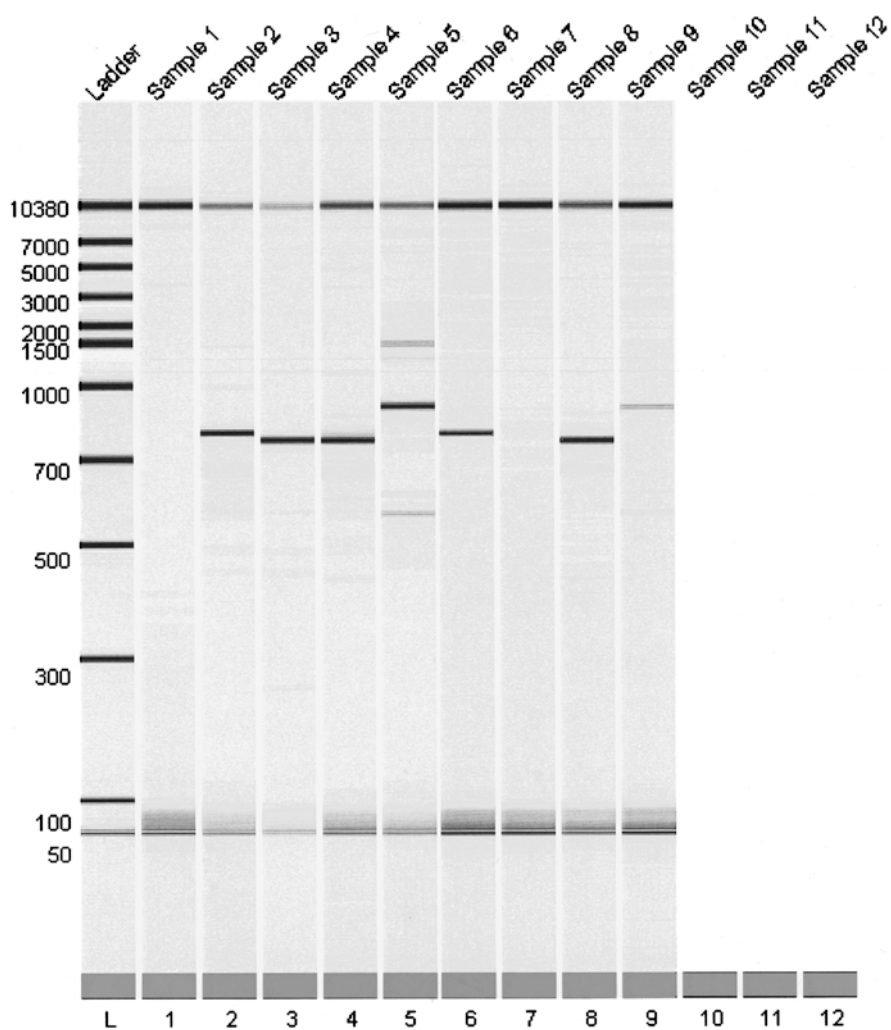


Photo 4 Identification of microorganisms found to occur in mineral dispersions.

Photo 3: Bacterial diversity in a CaCO_3 slurry sample from France 100x / light microscopy with phase contrast.

Figure 5: rDNA Internal Spacer Analysis (RISA) is a recently developed PCR technique that involves amplifying the spacer region between the 16s and the 23s genes of the rRNA operon. The length (and sequence) of nucleotides in this region is species specific, and can therefore be exploited to provide an insight into the bio-diversity of a sample. Most importantly, this can be achieved without cloning and sequencing, as a different sized PCR product is produced for each organism present in the slurry.

Photo 4: This gel shows RISA products, prepared by PCR amplification of the 16s-23s spacer region on the rDNA operon of several CaCO_3 slurry isolates. The number of bands corresponds to the number of rDNA operons inherent to each species while the size and sequence of each spacer region is species specific.

Depending on the nutrient supply, some species can be favoured. Similarly, in certain cases often found in mineral slurries, Methanotrophs and Methylotrophs can be favoured not only due to original nutrient sources but also in respect to the biocides used. A wide variety of bacteria are known that can grow on methanol, methylamine, or formate, and, in the case of Methanotrophs, also on methane. If, therefore, pure formaldehyde donors are used as biocide it cannot be ruled out that bacteria of these genera will accumulate over a certain period of time. This is just one example of how the spectrum of bacteria in relation to nutrient before and during preservation must be considered and not just the original dominant contamination.

The genera most frequently occurring in mineral dispersions, however, are the pseudomonads. These straight or curved gram-negative rods have very simple nutritional requirements and grow chemo-organotrophically at neutral pH and at temperatures in the mesophilic range. One of the striking properties of many species of pseudomonads is the wide variety of organic compounds that are used as carbon and energy sources. Some species of these genera also show a tendency to biofilm formation.

The gram-positive *Micrococcus* and *Staphylococcus* are both aerobic organisms with a typical respiratory metabolism. Gram-positive cocci are relatively resistant to reduced water content and have the potential to tolerate drying and high salt levels fairly well. Gram-positive cocci can most readily become introduced into a mineral dispersion via the addition of dispersants (salts of polyacrylic acid (PAA)). Usually, their growth is suppressed by the gram-negative bacteria that are dominant in the matrix, but this naturally depends on the stage of dispersant addition in the process and the prior existence or otherwise of dominant organisms.

An overview of the major microorganisms found to occur in mineral dispersions is shown in Table 1, although it has to be mentioned that only those species which could be repeatedly confirmed by the methods employed by the current authors have been listed, and, therefore, the list is by no means exhaustive.

5.8.5 Prevention and control of microbial activity – real time monitoring and evaluation

Prevention of contamination caused by microorganisms and the effects arising therefrom have economic consequences. These consequences must not be underestimated. For example, significant changes in the rheology of mineral dispersions can lead to a standstill in paper production. Similarly, continual false application of biocide(s) arising from an over-reaction philosophy can have equally dramatic economical effect.

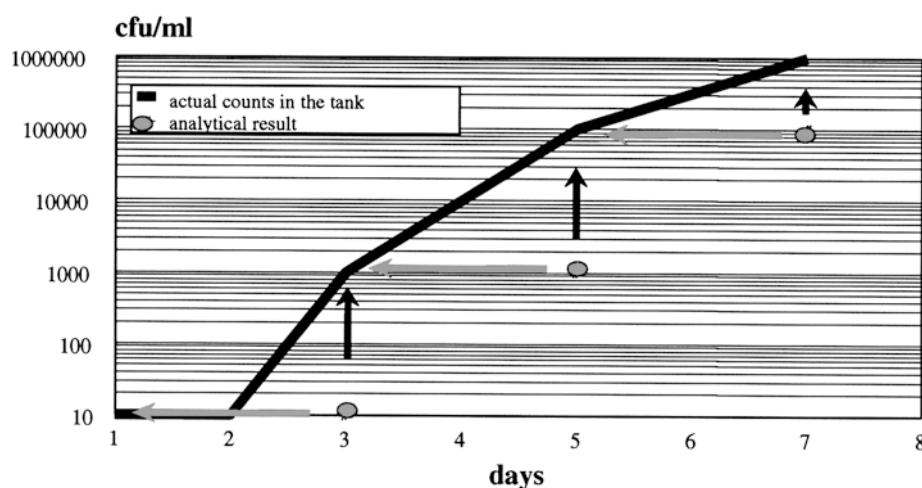
To achieve the goal of optimal control of microbial contamination, continuous monitoring of the biological activity in a system and well-balanced housekeeping (i.e. storage tank and transport cleanliness) are necessary. Together with monitoring, the process of optimal dosing and selection of biocide(s) becomes an integrated part of the strategy. It is therefore necessary to consider carefully the options available both currently and those under development for isolation and monitoring of the bacteria, as rapid identification and colony determination are crucial to the benefits that can be derived from optimising the control strategy.

Over the last years, classic methods for the determination of the total viable count have become established in the white mineral industry. The use of agar substrates in a Petri dish, or on a plastic film, are primarily suitable for a first count (and isolation) of aerobic or facultatively anaerobic microorganisms. This method can also be applied for not too extreme anaerobes provided the plates are incubated in an anaerobe jar. A significant disadvantage of this plate method for determining the total viable count is the long incubation time (culture enrichment) required (typically 48–72 hours) which strongly impairs the ability to apply quality control, and hinders online production security. Figure 6 clearly shows that, due to the long incubation times of traditional methods, the result does not reflect the current situation in the storage tank but the situation as it was some ~48 hours before.

New applications of established instrumental techniques, such as electronic cell counting, based on a Coulter-counter, as well as methods for vital counting (vital staining), offer interesting alternatives to the agar substrates. These options are reviewed briefly in the next section. Furthermore, we go on to consider novel methods of electro-optically identifying the bacteria undergoing vital staining.

Table 1 Microorganisms found to occur in mineral dispersions.

Group	Genus	Species
Aerobic/microaerophilic, motile, helical/vibrioid gram-negative bacteria	<i>Bdellovibrio</i>	<i>B. bacteriovorus</i>
Gram-negative aerobic/microaerophilic rods and cocci	<i>Acidovorad</i>	<i>A. delafieldii</i>
	<i>Agrobacterium</i>	<i>A. radiobacter</i>
	<i>Alcaligenes</i>	<i>A. xylosoxidans</i>
	<i>Flavobacterium</i>	<i>F. indologenes</i>
	<i>Methylobacterium</i>	<i>M. extorquens</i>
		<i>M. mesophilicum</i>
	<i>Pseudomonas</i>	<i>P. aeruginosa</i>
		<i>P. alcaligenes</i>
		<i>P. cepacia</i>
		<i>P. diminuta</i>
		<i>P. fluorescens</i>
		<i>P. luteola</i>
		<i>P. maltophilia</i>
		<i>P. mendocina</i>
		<i>P. paucimobilis</i>
		<i>P. pickettii</i>
		<i>P. pseudoalcaligenes konjaci</i>
		<i>P. putida</i>
		<i>P. stutzeri</i>
		<i>P. testosteroni</i>
		<i>P. vesicularis</i>
		<i>R. spp.</i>
Facultatively anaerobic gram-negative rods	<i>Rhizobium</i>	<i>S. spiritivorum</i>
	<i>Sphingobacterium</i>	<i>A. caviae</i>
	<i>Aeromonas</i>	<i>A. hydrophilia</i>
		<i>A. salmonicida achromogenes</i>
		<i>A. salmonicida masoucida</i>
	<i>Chromobacterium</i>	<i>C. spp.</i>
	<i>Vibrio</i>	<i>V. metschnikovii</i>
		<i>V. parahaemolyticus</i>
Budding and / or appendaged bacteria	<i>Hyphomicrobium</i>	<i>H. vulgare</i>
Sheathed bacteria	<i>Leptothrix</i>	<i>L. discophora</i>
Gram-positive cocci	<i>Micrococcus</i>	<i>M. luteus</i>
		<i>M. roseus</i>
		<i>M. varians</i>
	<i>Staphylococcus</i>	<i>S. capitis</i>
		<i>S. cohnii cohnii</i>
		<i>S. lentus</i>
		<i>S. sciuri</i>
		<i>S. xylosus</i>
Irregular, non-sporing gram-positive rods	<i>Agromyces</i>	<i>A. ramosus</i>

**Figure 6** Prevention and control of microbial activity.

Electronic cell counting: The Coulter-counter

The cells to be counted are suspended in a conductive aqueous solution, an electrolyte. An exactly determined small volume of this suspension is made to flow through a narrow capillary orifice which connects two chambers filled with the electrolyte. In each of these two chambers an electrode is immersed into the liquid. A current is flowed between the two electrodes, and the electrical resistance, which is generated by the narrow orifice, is measured. A biological cell passing the orifice leads to a temporary increase in the resistance as the electrical conductivity of the cell is much lower than that of the electrolyte. The voltage pulse generated by the increase in the resistance is amplified and electronically registered. In this way, the number of cells passing the orifice is obtained. Although this procedure enables the determination of the number of cells, it does not provide any information about the physiological state of the microorganisms. For this purpose, the cell suspension has to be transferred into a liquid culture medium and incubated.

Vital staining

In the case of ecological investigations, the customary methods for the determination of the number of cells do not show whether the detected microorganisms are physiologically active in the place where they are naturally occurring, only that they are active under the specific culture conditions employed. There is no doubt that the number of microorganisms with active metabolism in a mineral dispersion is considerably higher than the number found by the standard methods for the determination of the total viable count. On the other hand, it is often found that, in their natural habitat, a considerable number of the counted cells are in a state of rest and are physiologically inactive. For this reason, a series of staining methods have been developed by which it would be possible to recognise viable, metabolically active, microorganisms directly under the microscope, and to distinguish them from dead or inactive cells (Figure 7) (Fry, 1990; Hall et al., 1990; Lloyd & Hayes, 1995). Fluorescent dyes are preferably used for vital staining, as fluorescent cells are considerably easier to recognise and count than non-fluorescent ones.

Novel real-time monitoring and evaluation

To be able to react increasingly effectively to microbial contamination in the production of millions of tonnes of mineral slurries per year, and to optimise the biocide consumption costs, a method which measures directly the total viable count in real-time is urgently required. Such a method is nearing completion in its development.

A combination of the Coulter-counter and fluorescence techniques has been investigated and developed in application to mineral slurries by the current authors and is now seen to offer a potential solution.

Figure 8: Schematic view of the principle of CellFacts II[®]. Particles (or cells) in an electrolyte are sucked through a measuring orifice having a diameter of 30 µm. The laser (red or green) is focused at the measuring orifice.

With such a method the number of particles is counted and the volume determined in parallel. By staining the cells and determining the emitted fluorescence, the physiological state of the individual cells can be determined, so culture enrichment is no longer necessary. Without the need for enrichment, the result is given in real-time. This methodology is just emerging in practical field trials for mineral slurry contamination control.

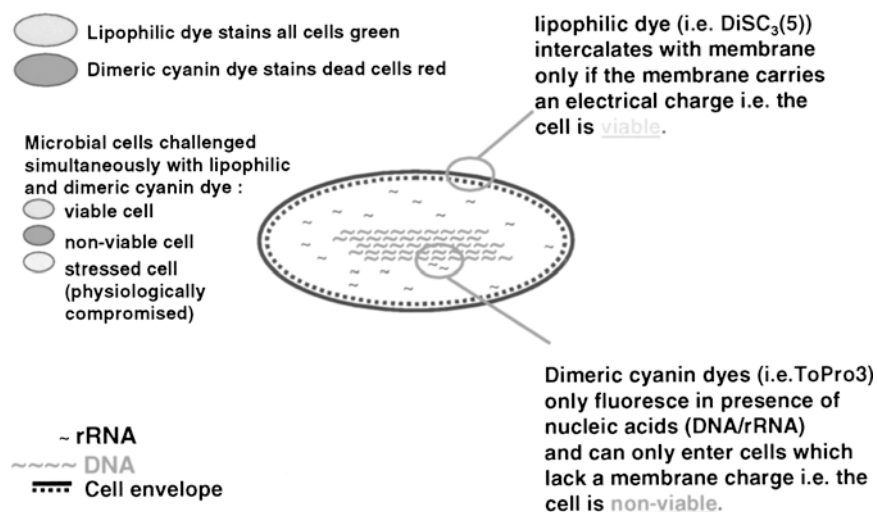


Figure 7 Vital staining.

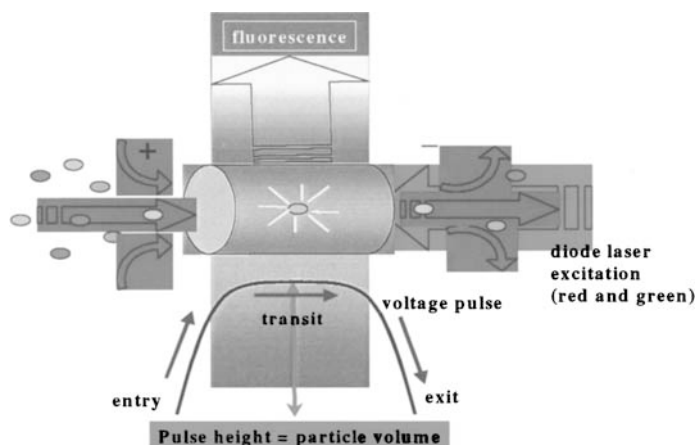


Figure 8 Schematic view of the principle of CellFacts II®.

5.8.6 Constraints on suitable types of microbicides

Today, biocides for the preservation of mineral slurries have to meet far more requirements than showing demonstrable bactericidal action alone. Some of the most important criteria which decide whether a bactericidal agent can be used for the preservation of mineral slurries can be summarised as follows:

- Must be thermally stable up to a minimum of 60°C.
- Needs to have a positive redox potential.
- Its inhibition of nitrification must be < 30% (in dilution) in slurry so as not to impair the nitrification in subsequent waste water purification plant.
- It must be biodegradable to a level greater than 80 w/w%, preferably 100 w/w% (OECD 301D).
- May not generate a negative inhibition area around finished paper or application surface - this applies to the need to prevent extraction of biocide from paper and packaging materials when in contact with foodstuff.
- Must have no negative influence on other mineral dispersion properties.
- Required to have Regulatory Approval (e.g. FDA, BgVV, Nordic Ecolabelling). See Chapter 4.

Among a large number of bactericidal agents the following ones have proved to be successful as preservatives in mineral dispersions. Once again, this list should not be considered as exhaustive: many biocides which have proven efficacy have either fallen out of favour due to regulatory requirements or even environmentally-related perceptions, and many biocides remain to be discovered or, if already existing, to be applied in this field. We go on to consider some currently used biocides and identify a newly applied biocide in this field.

2-Bromo-2-nitro-propan-1,3-diol (Bronopol) [II, 17.14.]*

Bronopol has a broad spectrum of antibacterial activity and belongs to the group of aldehyde-releasers as well as to the group of activated halogen-compounds. It is widely used as a preservative of pharmaceutical and cosmetic products (Croshaw et al., 1964; Storrs & Bell, 1983). Another important emerging application is the use as a preservative in the filler and pigment industry (e.g. calcium carbonate slurries).

A considerable disadvantage, however, is that the compound is not heat-stable.

At a temperature of 60°C under alkaline conditions the active agent is completely decomposed within a few hours.

Isothiazolin (MIT/CIT/BIT) [II, 15.]

Isothiazolin biocides, such as 1,2-benzisothiazolin-3-one (BIT) [II, 15.6.], 2-methyl-4- isothiazolin-3-one (MIT) [II, 15.1.] and 5-chloro-2-methyl-4-isothiazolin-3-one (CIT) [II, 15.2.] are widely used as environmental biocides as well as preservatives for filler- and pigment- systems.

Isothiazolinones have a high potential for sensitising (Weaver *et al.*, 1985) and microbicides based on isothiazolinones are not heat-stable (Willingham & Mattox, 1990). They are today mainly used in combination with bronopol or ethylene glycol hemiformal [II, 3.1.4.] for preservation in the pigment and filler industry.

*see Part Two – Microbicide Data

Phenol derivatives (e.g. o-phenylphenol) [II, 7.]

Phenol [II, 7.1.], also termed carbolic acid, and the phenol derivatives could also be considered as acids because of their acidity and the resulting capacity to form stable salts. Carbolic acid and soaps have been widely used as cleansers and disinfectants including in the home and in medical environments. Today a wide range of phenols is used for the protection of materials. Since o-phenylphenol [II, 7.4.1.] is used to preserve citrus fruits because it has the most favourable toxicity data (Paulus, 1993) this phenol derivative especially has rapidly gained importance as a preservative for the protection of pigment slurries, and this is new. It is, however, not ideal as a disinfectant in slurries due to the relatively low solubility level, although its value in preservation is being established; its application forms in fact being under current patent rights.

Aldehydes (e.g. glutaraldehyde) [II, 2.]

Aldehydes have a broad spectrum of activity. Bacteria, fungi, spores and viruses are killed or inactivated. This, however, depends on the effective or differing exposure times.

The pressure from the pigment and filler industry to abstain from using formaldehyde [II, 2.1.] is increasing. Considering that 200 ppm per tonne pigment/filler have to be used for a sufficient preservative action, it is clear that in the production of paper (wet end), quantities of formaldehyde can become released. Due to the high vapour pressure of formaldehyde, vapour is frequently released into the air during and after the disinfection or preservation procedure and concentration levels can build-up in enclosed production environments. Furthermore, this active agent has been classified as sensitising.

Formaldehyde-releasing compounds (e.g. ethyleneglycol-hemiformals) [II, 3.]

Formaldehyde, as such, is often too volatile and too reactive to be used as a microbicide for the protection of white minerals. It additionally produces unwelcome side-effects such as an increase in viscosity, and has an insufficiently balanced range of activity. One, therefore, continually searches for formaldehyde-releasing compounds which do not exhibit the disadvantageous formaldehyde effects but maintain or even improve the antimicrobial action of formaldehyde. However, the environmental limitations on released formaldehyde still apply.

Formaldehyde-releasing compounds can be found as both solids and liquids, water soluble or oil soluble, alkaline, neutral, or slightly acidic. According to their composition, the ethylene glycol-hemiformals [II, 3.1.4.] are especially effective against bacteria and therefore useful for the so-called “in-can”, or storage container, protection of a large variety of industrial fluids, mainly together with other active ingredients, e.g. fungicides. Also, the protection of white mineral slurries using ethylene glycol-hemiformal has proven to be effective.

3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione (DAZOMET) [II, 3.3.25.]

3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione has a broad spectrum of high activity which covers bacteria, fungi and yeast, indicating that the substance is a specialised formaldehyde-releasing compound.

The spectrum of activity is attractive for application in a number of industrial systems, e.g. as a broad spectrum microbicide which prevents fungal blooms in metal working fluid systems. However, there are limitations, including the finding that 3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione is very unstable at increased temperatures. Under alkaline conditions it is found that more decomposition products are formed than under pH neutral conditions (e.g. wet-end system).

The intermediate breakdown products formed under alkaline conditions have a strong smell. The preservation of filler and pigment slurries with thione has a high risk for bad smelling slurries and end product, such as paper, especially when heated in a humid environment.

2,2-Dibromo-3-nitrilopropionamide (DBNPA) [II, 17.5.]

DBNPA has a broad spectrum of high activity with a low environmental impact which covers bacteria, fungi and yeast.

Such microbicides are fast acting, but are temperature sensitive and will decompose exothermally (liberating heat) at elevated temperatures. They are today mainly used in combination with other biocides, such as bronopol or ethylene glycol hemiformal for preservation in the pigment and filler industry.

Methylene bithiocyanate (MBT) [II, 20.9.1.]

MBT is an effective microbiocide for bacteria, algae, yeast and fungi, but has a limited stability in particular at pH values > 7.5. The microbiocide must preferably be added at a point of good agitation to a storage tank with adequate turnover.

5.8.7 Regulatory, safety and environmental issues – some practical points (see also Chapter 4)

Since mineral dispersions might be used in the production of food packaging the constituent substances have usually to comply with most of the relevant laws, such as BfR Germany, FDA USA and current and emerging EU directives.

With respect to the relevant laws in different countries, not only the quality of the biocides but also possible limitations of the quantity have to be considered. Special attention has to be paid to the fact that different countries may also impose different limitations on the quantity. Below, we identify some of the most relevant criteria applicable today.

Biocidal product directive 98/8/EG

The aim of the Biocidal Product Directive 98/8/EG, passed in February 1998, is to harmonise the marketing of biocidal products (this includes products such as wood preservatives, disinfectants, pesticides and antifouling paints) within the EU. This results in comprehensive new regulations being laid down so that the supply of active substances will continue to be ever more severely restricted.

Risk for humans and environment

The requirements with respect to the toxicity of an antimicrobial substance primarily depend on the intended use. The most rigid requirements have to be met by products used for food preservation as people are exposed to high concentrations of them (0.05–0.50%) over a long period of time, often daily. For this reason it is most important that these substances are recognised as safe for health.

With respect to the intended use it is necessary to consider not only the quality of the biocide but also possible limitations of the quantity especially with respect to biodegradability, air pollution, by formaldehyde for example, and the risk of skin-sensitisation for humans. To review this area of legislation is beyond the scope of this article.

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5.9 Protection of cosmetics and toiletries

R. SCHOLTYSSEK

5.9.1 Introduction

It is generally acknowledged that water-containing products, including many cosmetics, can support the proliferation of micro-organisms. Without sufficient preservation, this in turn can lead to product spoilage, which in cosmetic products may be manifest as changes in smell, discoloration, mould growth, gas formation, the separation of emulsions or changes in viscosity, rendering the product unacceptable to the consumer. Such obvious spoilage invariably results in substantial financial damage and loss of image for the cosmetics manufacturer. Furthermore, non-visible microbial contamination also presents a significant danger, posing a risk to health should the micro-organisms be potentially pathogenic. Cosmetic manufacturers are themselves responsible for the safety of the products they produce and market. They are legally bound to ensure their products, if used as instructed or in any foreseeable manner, pose no risk to consumer health.

The following chapter outlines numerous measures which, if implemented by efficient and effective quality management, are an invaluable aid to ensuring the manufacture of 'safe' cosmetics products. The basis of a product's microbiological stability is established during the development phase, by selecting an appropriate preservative system subject to a comprehensive programme of validation checks. However, this alone is no sufficient guarantee of end-product stability if not supported by adequate hygiene during the entire manufacturing process. Under no circumstances should a product's preservative system be considered as a catch-all 'safety net' compensating for shortcomings in production hygiene. On the contrary, sound production hygiene is an essential complement to an effective preservative system selected during product development, ensuring stability during storage and consumer safety during use.

Biocides are used in some cosmetic products, not only as preservatives, but to fulfil special product-specific functions: in anti-dandruff preparations to combat the micro-organisms that aggravate dandruff; in deodorants to inhibit the growth of the micro-organisms responsible for unpleasant body odour; in dental and oral hygiene products to fight plaque formation; as antibacterial agents in skin cleansing products for treating juvenile skin problems; and in antibacterial hand washing products. Since the anti-microbial agents used in these products also contribute to total product preservation, cosmetic products with special anti-microbial functions are also discussed in this chapter.

5.9.2 Description of cosmetic products

5.9.2.1 Definition

Cosmetics are products people use to enhance and care for their outward appearance. Definitions as to what exactly constitutes cosmetic materials, medicines and foodstuffs vary somewhat from country to country, giving rise to the contentious expression, "cosmeceuticals", a term which underlines the sometimes close relationship between some cosmetics and pharmaceutical products.

The European Union (EU) distinguishes between cosmetics and medicines, biocides, foodstuffs and consumer articles. EU Directive (EC, 1976) defines cosmetic materials in (76/768/EEC), Article 1, Paragraph 1 as: 'A "cosmetic product" means any substance or preparation intended for placing in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or principally to cleaning them, perfuming them or protecting them in order to keep them in good condition, change their appearance or correct body odours.'

This definition targets products used in the direct treatment of the human body's external surfaces. According to EU regulations it includes products used to combat halitosis, or for oral and dental care, (e.g., chewing tablets or chewing gum; Zipfel & Rathke, 2001), yet excludes preparations intended for internal use as skin nutritional supplements, such as tablets, capsules or drops.

5.9.2.2 Important product classes

Products at low risk of microbial spoilage, i.e., those effectively free of water, strongly acid or alkaline, containing strong oxidizing or reducing agents, or a high alcohol content are not considered in this account. This chapter is primarily concerned with water-containing products susceptible to micro-organisms growth such as emulsions, gels, aqueous dispersions, surfactant products and sticks. Examples of typical cosmetic products are listed in Table 1.

Table 1 Typical cosmetic products

Category	Cosmetic material
Face cleansing	washing lotions, peeling, eye make up remover, face water, cleaning wipes and pads.
Face care	day and night creams, lotions, masks.
Shaving materials	pre- and after-shaves, shaving soaps.
Body cleansing	shower and foam baths, bath accessories, soap bars, liquid soaps.
Body care	creams, lotions, oils, hair-removers.
Intimate body care	wash lotions, deodorants.
Foot care products	creams, lotions, deodorants.
Sun protection and care products	emulsions, gels, oils, lipstick, after-sun emulsions and gels.
Deodorants and Antiperspirants	emulsions, powders, sticks, sprays.
Oral and dental care products	toothpastes, creams, mouthwashes.
Hair cleansing	shampoos.
Hair care	treatments/conditioners.
Hair styling	gels, hair setting lotion, sprays, permanent wavers
Hair colouring	cream and liquid hair dyes, toners, highlighters, bleaching agents.
Fragrance	perfumes, toilet waters.
Decorative cosmetics	lipsticks, eye liner, mascara, make up, nail polish.

Emulsions (Eigener, 1995). Emulsions form the basis of skin and face care creams, salves and lotions, but are also used in manufacture of hair conditioners and treatment preparations, roll-on deodorants, shaving creams, sun protection preparations, after sun care products and some decorative cosmetic products. Emulsion are mixtures of water and oil phases, stabilized by emulsifiers, in which either water droplets are suspended in an oil phase (W/O emulsion), or vice versa (O/W emulsion). Phase status has an important influence on the susceptibility of an emulsion to microbial proliferation, since preservatives and other supplements intended to act against the growth of such organisms often display an intrinsic preference for either the oil or aqueous phase, depending on their hydro- or lipophilic nature.

W/O emulsions are generally less vulnerable to micro-organisms than O/W emulsions. This can be attributed to two factors: firstly, the continuous oil phase tends to prevent contact between the water droplets of the discontinuous phase and micro-organisms, and secondly, the small size of the water droplets itself hinders the survival and proliferation of any microbes that may be present. Current emulsion technology can achieve an average water droplet diameter of 1 μ , approximately that of individual coccoid bacteria. In micro-emulsions, droplet size is even smaller. In contrast, manufacturing O/W emulsions with such characteristics, and thus dispensing with the need for chemical preservatives, is particularly difficult and requires special 'know-how' of emulsion chemistry.

Cleansing products. Skin and hair cleansing products, e.g. shampoos, shower and bath products, hand and face lotions are generally based on anionic surfactants. Eigener (1995) has comprehensively described the composition of these product groups. To improve skin compatibility, they generally include supplements, such as amphoteric and sugar surfactants, hydrolysed proteins, and other special extracts. Further supplements include foam stabilizers and thickeners. While cationic surfactants exhibit good cleaning performance, they foam less well than anionic surfactants, and are used in shampoos as conditioners to make combing wet hair easier.

Electrolytes, alkanolamides and cellulose derivatives are used as thickening agents. To prevent skin drying, humectants (polyalcohols and glycerin) and refatting substances (oils) are used. Preservatives, plant extracts, anti-dandruff agents, pearlescent, fragrances and colours are variously added as supplementary raw materials.

The cleansing product group also includes a number of preparations with special anti-microbial functions:

- anti-dandruff preparations, intended to combat the yeast, *Pityrosporum ovale* (*Malassezia furfur*) responsible for aggravating dandruff.
- anti-microbial hand wash preparations, used for both cleansing and combating undesirable micro-organisms.

5.9.2.3 Special products of microbiological relevance

Products for juvenile skin problems. Products for treating juvenile skin problems belong to both product categories (Siemer, 1991). In addition to their main function of skin care or cleansing, they are designed to combat pustule-forming bacteria in the skin. In the case of acne, these include Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermis* and *Propionibacterium acnes*. Antibacterial agents added to these products (5.9.4.3./preservatives) selectively aim at such organisms.

Deodorants/antiperspirants (Eigener, 1995). Another group of products are known as deodorants and antiperspirants. A deodorant is designed to both absorb and mask body odour with fragrance, and to inhibit the bacterial metabolism responsible for breakdown of sweat and subsequent production of unpleasant body odour. Antiperspirants suppress the release of sweat, using astringents such as aluminium hydroxychloride. Deodorant products and antiperspirants are manufactured in a number of different forms, including powders, sticks, roll-on products (emulsions), sprays and gel products. An important means of combating body odour is the use of anti-microbial agents (5.9.4.3.) which act selectively against the bacteria responsible for unpleasant body odour. To avoid disrupting equilibrium of the skin's native flora, the objective is to merely inhibit growth of target bacteria (bacterio-static effect; Bremer, 1991). As a general rule, all preservative substances expressing selective efficacy against Gram-positive bacteria can be considered microbe inhibitors. In addition to preservatives, many aromatic substances display antibacterial efficacy, but their strong smell or allergenic potential limit their use in such cosmetics. Another method of preventing body odour is the use of enzyme inhibitors, which inactivate the ester-cleaving lipase enzymes responsible for the breakdown of sweat without affecting the bacteria producing them.

Toothpastes (Hellwege, 1991). The abrasive and polishing function of toothpastes is served by their relatively high inorganic content (hydrated silicas) and this cleaning action is increased by the addition of surfactants. Aromatic substances are added to confer a pleasant taste and further additives include thickening agents, colouring agents, and humectants. The moist environment of the oral cavity provides almost ideal conditions for the growth of many bacteria. If inadequately cleaned, bacteria lodged in the dental and gingival interstices undergo a rapid increase in numbers. The surface of the teeth themselves also provides an ideal adhesive surface for the accumulation of bacterial deposits (plaque): between 200–300 superimposed layers of bacteria of various types can develop within a few days of initial colonization. Further rapid growth of this diverse bacterial colony causes plaque to spread across the tooth into the gums, giving rise to infections. To combat plaque formation and bacterial growth, dental and oral care products contain anti-bacterial agents.

5.9.3 Microbiological safety of cosmetic products

5.9.3.1 Microbiologically susceptibility

Due to the nature of their composition, many cosmetic products are vulnerable to decomposition by micro-organisms. Biologically available water, appropriate nutrients and temperate micro-environmental conditions (e.g., equitable temperature, pH) favour the increase of micro-organisms, and cosmetics often need protection against spoilage. The intrinsic, self-preservative properties of a formulation, such as physical-chemical effects (5.9.4.2.), or raw materials with secondary anti-microbial efficacy (5.9.4.3.) may be sufficient, without recourse to the use of chemical preservatives. However, with aqueous products adequate preservation is often only possible with the addition of chemical preservatives. A preservative system should ideally protect the product from microbial spoilage both in its unopened container up to the guaranteed use-by-date, and in an opened container for the duration of its use. However, the capacity of product preservation has limits. Thus it is imperative that all microbial influences liable to adversely affect the product, other than those unavoidable encountered during consumer use, are reduced to an absolute minimum. In effect, this means, all potential sources of contamination need to be identified and eliminated for the entire process of manufacture, from the delivery and receipt of raw materials to dispatch of the end-product. Guidelines for good manufacturing practice of cosmetic products (CGMP, 1994; GMPC, 1995) are discussed later, in the section on plant hygiene (5.9.6.).

5.9.3.2 Causes and consequences of microbial contamination

The causes of microbial spoilage may be both diverse and multifactorial: the quality of product preservation, the microbiological quality of the raw materials used - in particular production water microbe content, packaging and manufacture, and user contamination.

Raw material contamination. In the broader context of product preservation, attention needs to be paid to adequate raw material preservation. Cosmetic raw materials and raw material mixtures need protection against microbial contamination during their transport, storage and use in production. Contaminated raw materials introduced into production can severely load, or even over-load, a product's preservative capacity such as to render it ineffective. Raw materials are derived from synthetic, animal, plant and mineral sources. Depending on their origin and processing, they may to a lesser or greater extent be either already contaminated or susceptible to subsequent microbial contamination. Water-free raw materials of synthetic origin generally pose few microbial spoilage problems. Similarly, water-free synthetic oils, waxes and fats, as well as emulsifiers, concentrated surfactants and surface-active agents are unlikely to support the growth of micro-organisms. This reassuring state of affairs changes dramatically as soon as they are mixed with other aqueous ingredients.

Even natural raw materials supplied as water-free powders or granulates may harbour micro-organisms, viruses, prions, or microbial toxins. Analysis of such materials may well reveal bacteria, clostridian spores, staphylococci, moulds and in particular fungal toxins. Furthermore, the possible presence of bacterial spores cannot be ruled out, since they may even be present in preparations with a high percentage of alcohol. Natural raw materials extracted, processed or supplied as aqueous preparations are particularly susceptible to microbial contamination. Insufficiently preserved, these raw materials can support the growth of Gram-negative micro-organisms such as *Enterobacter spp.*, *E. coli*, *Citrobacter spp.*, *Pseudomonas spp.*, etc., when used to manufacture solutions, dispersions and emulsions.

Product contamination. Bacteria and moulds reveal their presence in contaminated cosmetics in numerous different ways. They metabolize the various ingredients of cosmetic products, and so cause product spoilage. In practice, some cases occur where a product equipped with an apparently adequate preservative protection nevertheless supports the growth of particular micro-organisms. In such cases, these micro-organisms clearly show reduced sensitivity to the preservative system employed, in other words they have adapted to the incumbent antimicrobial parameters. Such adaptation is often provoked by preservative under-dosing, and the progeny of survivor micro-organisms show a distinctly increased resistance to the surrounding preservative milieu. However, such micro-organisms lose their adaptive sensitivity when sub-cultured under laboratory conditions on the usual nutrient media. This has important consequences for laboratory analyses if such strains are used for preservative challenge testing (5.9.5.2.). In contrast to strains displaying reduced sensitivity, the genuine resistance behaviour of micro-organisms is non-reversible.

Functional product damage. Product spoilage can take many forms, for instance as visible growth on the surface of the product (e.g., moulds, micro-organisms producing pigmentation), whereas with washing and cleansing materials the commonest symptom of contamination is a change in smell, caused by the release of sulphur compounds, but may also include discoloration and partition of pearlescent agents. Spoilage can invariably be traced back to enzymatic breakdown of the product's ingredients. Many enzymes remain active, even when the micro-organisms that produced them have been inactivated, and thus even the use of microbe-reduction measures may not unequivocally rule out the chance of product spoilage.

In addition to causing product spoilage (Table 2), microbial contamination can also pose a risk to health.

Table 2 Spoilage causing micro-organisms

Organisms	Cause of product defects
Gas formation (bombages)	
Clostridia	carbon dioxide, hydrogen
Lactic acid bacteria, Yeast	carbon dioxide
Enterobacteria	carbon dioxide, nitrogen, hydrogen
Denitrifiers	nitrogen, nitrous oxide
O ₂ -consumers	
All aerobic micro-organisms	deformation of packaging
Alteration in pH	
Fermentative acid formation	
-Clostridia	butyric acid
-Lactic acid bacteria	lactic acid, acetic acid
-Enterobacteria	formic acid, acetic acid
-Yeast	acetic acid, lactic acid
pH increase by protein degradation	
-Clostridia	ammonia
-Pseudomonads	
Alteration of colour	
Sulphate formers	sulphides
Pigment formers	
Alteration of viscosity	
Slime formers	increase of viscosity
Degradation of polymers	decrease of viscosity
Lipolytes	decrease of viscosity
Separation of Emulsions	
Different micro-organisms	by degradation of emulsifying agents
Fermentative micro-organisms	by formation of alcohols
Alteration of smell	
Sulphate reducers	hydrogen sulphide
Protein degraders	amines, hydrogen sulphide
Fermentative organism	acids, alcohols
Lipolytes	rancidity
Visible growth of micro-organisms	
Moulds	colonies
Slime formers	flocks of slime

Product contamination and risk to health. Contamination is not always manifest solely as product spoilage. Kallings et al. (1966) published a report on non-sterilised medicines, which although showing no obvious signs of spoilage, contained sufficient levels of pathogenic micro-organisms to represent a significant risk to health. Similarities between the formulation of cosmetics and medicines has led to the suggestion of an analogous health risk for cosmetics. Such a health risk may be particularly acute in the case of contaminated products used on injured skin, the oral cavity and respiratory systems, the eyes, on babies where the skin barrier is not fully developed, or people with a compromised immune system. Baird (1984) described infections in new-born babies, attributed to *Pseudomonas aeruginosa*, *Klebsiella*, *Serratia*, *Clostridium perfringens* and *Clostridium tetani*. Pseudomonad infections involving eye products have been frequently observed.

Practical relevance of health risks in cosmetics contaminated with micro-organisms. The microbiological risk associated with contaminated products depends on the number and type of the micro-organisms involved, and the toxins present. Numerous reports on microbial contamination in cosmetics have been published in Europe. Eigener (1995) evaluated these studies and referred to a publication from Doorne (1992) that reported on a study of cosmetic materials in the Netherlands from 1985 to 1989. Of the 2430 products examined, 7.8% revealed a micro-organism count $>10^3$ cfu/g. The majority of cases involved Gram-negative bacteria, in particular pseudomonads. In another study from Great Britain, Baird (1977) investigated 147 retail cosmetic products, of various types, of which 67.3% were free of bacteria. 23 samples contained sporogenous aerobic bacteria and 20 samples Gram-positive coccoid bacteria (extent of microbial contamination is not stated). 6.1% samples contained Gram-negative bacteria, mainly pseudomonads and enterobacteria.

Despite these reports of microbial contamination in cosmetics, since the mid-70s in the EU, there is little hard evidence of contaminated cosmetics causing infections in consumers. Possibly this is because the risk to health associated with external application of a contaminated product should be rated as rather low: the mere presence of pathogenic micro-organisms itself is not sufficient to trigger an infectious process. Nevertheless, contamination by pathogenic micro-organisms should be taken seriously, and measures be taken to avert any potential risk to health in good time. Should a single instance of microbial or toxin contamination be confirmed in a product, then a specific risk assessment of the possible influence on the consumer's health has to be made. The risk potential listed in Table 3 could form the basis of such a risk assessment, although each case requires consideration of product- and use-specific risks.

5.9.3.3 Official regulations

Industry association regulations: CTFA/CTPA. In 1973, the US Cosmetic Toiletries Fragrance Association (CTFA) and the UK Cosmetic Toiletries Perfumery Association (CTPA) published maximum permitted levels of micro-organisms for different product classes and issued requirements for the exclusion of potentially pathogenic micro-organisms. Table 4 lists the permitted maximum levels of micro-organisms and well-known representatives of pathogenic organisms. Good manufacturing practice guidelines for cosmetic products have been drawn up as practical recommendations for the improvement and maintenance of manufacturing hygiene (CGMP, 1994; GMPC, 1995). They clearly state that preservatives should only be used for to protect the formulation in storage and in use, and should never be used to compensate for inadequate production hygiene. Both associations have also published methods for running preservative challenge tests, as guidelines for validating the efficacy of preservatives used in product preservative systems.

Table 3 Microbial risk factors for human health by contaminated cosmetics based on data by (Heinzel, 1999b)

Organisms	Possible symptoms
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	pus, sepsis
<i>Streptococcus pyogenes</i>	ditto
<i>Enterococcus spp.</i>	infections
<i>Clostridium tetani</i>	tetanus
<i>Clostridium perfringens</i>	gas gangrene
Gram-negative bacteria	
<i>Pseudomonas aeruginosa</i>	conjunctivitis, pus, infections
<i>Klebsiella spp.</i>	conjunctivitis, infections
<i>Enterobacteriaceae</i>	enteritis
Fungi	
<i>Candida albicans</i>	conjunctivitis
<i>Candida parapsilosis</i>	conjunctivitis
<i>Malassezia furfur</i>	dermatomycosis
<i>Trichophyton spp.</i>	dermatomycosis
<i>Trichoderma</i>	inflammations
<i>Aspergillus spp.</i>	allergic reactions

Table 4 Microbiological acceptance criteria for cosmetics and toiletries based on data by (Hill, 1995)

Product category	Microbiological limit (*cfu/g or cfu/ml)	
	CTFA ¹ (US)	SCCNFP(EU)
Baby products	500	100 ^{2a}
Products used around the eye EU also mucous membrane	500	100 ^{2a}
Products for general use	1000	1000 ^{2b}

*cfu: colony forming units.

¹In addition to the microbial limits specified above, no product shall have a microbial content recognized as harmful to the user as determined by standard plate count procedures.

Specific micro-organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

^{2a}*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* not detectable in 0.5 g or ml.

^{2b}*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* not detectable in 0.1 g or ml.

Regulations in the EU. The European Economic Community Cosmetics Directive (76/768 EEC), under the auspices of Consumer Protection 1976, published binding directives for the manufacture of cosmetic products (EC, 1976). Article 2 of these guidelines requires that cosmetic products must not be injurious to the consumer's health under normal conditions of use, with responsibility borne by the product's manufacture. To protect consumers from possible toxicological risks caused by the use of preservative materials, a list of preservatives was published in annex VI of the directive: those in List A were given clearance, whilst those contained in List B were given provisional clearance, in other words not yet subject to conclusive evaluation. In the January 2000 edition, only definitively permitted substances are listed. This list defines, in addition to substance class, the maximum permitted concentration, specified conditions of use and instructions for warning labelling.

Micro-organism limits (see Table 4) are listed in guidelines published in Annex VIII of the SCCNFP/031/00 Final (The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers). They also reflect the dermatological and toxicological safety of cosmetic materials.

Cosmetics with anti-microbial claims. The evaluation and proof of safety of cosmetic products includes not only consideration of a product's microbiological stability, but also any additional claims of anti-microbial efficacy. Such claims have to be proven, documented and then checked against the appropriate regulations for cosmetic products. Depending on the claims made, such products often are borderline cosmetics, pharmaceuticals or disinfectants. In some countries, products are accredited as pharmaceutical substances if they claim to be effective against pathogenic micro-organisms, and prophylactic use of the product is recommended as protection against infection.

In the EU (EC Directive 76/768) a product is considered a cosmetic as long as its intended use is as a care or protective product, and it makes no illness-specific claims and is not used internally (Heinzel, 1999b). Products claiming an antiseptic effect (medical claim – against sepsis) are not considered cosmetics, but pharmaceutical products (EEC, 1965). Disinfectants are either classified as medical products (EEC, 1993) or fall under the jurisdiction of biocide guidelines (EC, 1998).

Section 5.9.2.3. has already described cosmetic products which claim additional anti-microbiological effects. Deodorants are designed to inhibit bacteria whose metabolism is responsible for producing unpleasant body odour, whereas products for the treatment of juvenile skin problems are intended not only to cleanse the skin but also inhibit the growth of pustule-forming bacteria, etc. All these products are either equipped with anti-microbial agents selected to target the offending micro-organism, or the concentrations of the preservatives used for product protection are increased. Proof of effect must match product claim, and it is important to consider whether the statement of claim is for selective inhibition of bacteria (bacteriostasis), mould (fungistasis), or micro-organisms generally (microbistasis), or micro-organism reduction/death (bactericide, fungicide, microbicide). Furthermore, the user expects that efficacy claims be fully realised within the time-span claimed in the conditions for use. Therefore, test design must match the product under test: a test for mouthwash requires a different test design than that for deodorants where the product remains on the skin for hours. In the EU, no official evaluation standards currently exist for minimum efficiency time with respect to inhibition or a lethal microbial effect. However, procedures for testing anti-bacterial, anti-mycotic or anti-microbial effects are laid down by various EU and US (ASTM, Amer. Soc. for Testing and Materials) norms (e.g., EN 1276, EN 1650, EN 1499, EN 1500 and ASTM E1174, ASTM E1327). Test conditions (test micro-organism; treatment conditions, time, temperature, load, etc.) can and should be realistically adapted to the conditions of use.

In the USA, products with anti-microbial claims are sold as over-the-counter (OTC) drugs. This may include cosmetics, with supplementary uses as infection inhibitors, or with healing and palliative (soothing) properties. In contrast to US pharmaceutical products, OTC products may not contain active agents listed in the US Pharmacopoeia, National Formulary or Homeopathic Pharmacopoeia.

Monographs are published in which OTC drugs are comprehensively described and contain a list of recommended claims and a positive list for useable active agents. As long as manufacturers adhere to these instructions, they need not register the product: it is automatically recognised as being GRAS/E (generally regarded as safe and effective). However, should claims or active agents other than those described in the monographs be used, then the product has to be registered anew, as “new drug application (NDA)”.

5.9.4 Protection of cosmetics from microbial spoilage

The following deals in particular with microbe-susceptible, water-containing raw materials and formulations. As a rule, water-free products lack the conditions necessary for micro-organism proliferation and are not discussed further in this chapter.

5.9.4.1 *The concept*

As already stated, the need for microbiological cleanliness in the manufacture of cosmetics is paramount. Even if a product is manufactured free of micro-organism contamination, during consumer use it will almost certainly be exposed to a more or less large number of micro-organisms unless securely packaged against contamination, either as a single-use product or in a gas-pressurized container. For example, the large exposed surface area of a wide-mouthed pot or jar of face cream, open to the atmosphere and in repeated contact with the more or less heavily contaminated hand of the user, presents a scenario highly favourable to post-production microbial contamination. Despite this potential for contamination, micro-organisms introduced to the product in this way should neither pose a potential risk to user health, nor result in product spoilage. If the intrinsic properties of the formulation are not capable of destroying these micro-organisms, or reducing their number, then it should at least exercise a microbiostatic effect to prevent microbe proliferation. A formulation's preservative properties influence micro-organism metabolic activity, and when effective can halt metabolism, in other words, effect bacteriostasis or fungistasis, or even death of the micro-organism. Preservation can either be produced by ensuring unfavourable physical-chemical conditions for microbial growth prevail and the formulation possesses intrinsic, self-preservative properties, or by adding chemical preservatives. The concentration of perfume oils, chemical preservatives and plant extracts with manifest preservative properties to effect intrinsic formulation preservation require optimisation, as relatively low concentrations of these substances are known to be poorly tolerated by the skin. Cosmetic product preservation requires careful planning and a regime of thorough checking, taking into account the following points:

- *Preservative protection.* This involves selection of raw materials that act against micro-organism proliferation, and/or the selection of chemical preservatives benign to both other formulation ingredients and packaging materials. Furthermore, the concentrations used must be verifiably compatible with the skin and yet provide sufficient protection for the formulation.
- *Primary packaging.* When planning primary packaging for a product, the role packaging design and form plays in the risk of potential exposure to micro-organisms needs careful consideration, (i.e., narrow mouth tube vs. wide mouth pot).
- *Testing preservation.* Preservation testing can be done by artificially contaminating a product with microbes in the laboratory, simulating the likely microbial challenge experienced during consumer use (microbial challenge test). In some cases, laboratory results of such tests require confirmation from so-called “home use tests”, whereby the influence of not only user-behaviour, but also the prevailing environmental conditions (e.g., warm, damp bathroom) on the product protection is tested. Simple application tests in the laboratory are no substitute for “home use tests”.

5.9.4.2 *Preservation using physical and chemical factors*

a_w value. Micro-organisms require biologically available water for growth. The total water content of a formulation may not necessarily be the benchmark for determining the microbiological susceptibility of a formulation if it contains substances capable of binding water. Biologically available water, a_w (water activity), is defined as the relationship between the vapour pressure of a substance or formulation and pure water at the same temperature, whereby the a_w of pure water is 1.000. Table 5 lists a_w limits for various micro-organisms. While yeasts and moulds tolerate a somewhat lower a_w , Gram-negative bacteria require values in excess of 0.91 (Leech, 1988). The amount of freely-available water in cosmetic formulations can be reduced by water-binding substances such as glycol, sugars and salts etc. In this way, soap cake or bars, surfactant formulations such as shampoos (Curry, 1985), shower gels and toothpastes can be adequately preserved without the addition of anti-microbial preservatives. In practice, an a_w of <0.8 is generally considered to guarantee sufficient protection.

Table 5 Water activity limits for microbial growth

Organisms	a_w Limits
Gram negative bacteria aerobic spore formers	0.95–1.00
Staphylococcus Lactobacilli	0.90–0.95
Common yeasts	0.87–0.90
Common moulds	0.80–0.87
Halophilic bacteria	0.75–0.80
Xerophilic moulds	0.65–0.75
Osmophilic yeasts	0.60–0.65

Redox potential. Redox potential (E_h) is an index of the oxidative or reductive efficacy of a formulation and is calculated by the Nernst equation, as the ratio of oxidised to reduced substances in a substrate. The relevance of redox potentials in biological processes has already been documented (Kunz, 1994). Aerobic organisms generally require a relatively high E_h value (+100 to +500 mV), facultative anaerobic micro-organisms in contrast, tolerate both positive and negative E_h values. Anaerobic micro-organisms require very low E_h values (–200 to –400 mV) for growth. As a rule, cosmetics that contain strong oxidising agents (hair bleaching agents) or reducing agents (depilatory agents) require no additional preservatives.

pH values. The pH of a formulation can also strongly influence the growth of micro-organisms. Although in the natural world, some micro-organisms exist in extremes of environmental conditions, extreme pH can be used as a preservation strategy in cosmetics as long as there is no evidence of microbial adaptation. For example, high pH produces adequate protection in some types of aqueous raw material (e.g. surfactants), or stabilizes oxidation hair colours. However, compatibility problems preclude the use of extreme pH as a preservative method for products intended for direct application to the skin.

5.9.4.3 Influence of cosmetic constituents on product preservation

The composition of a cosmetic product determines its susceptibility to microbial adulteration. Cosmetic products are manufactured from animal, plant, mineral and synthetic raw materials. Depending on their chemical structure, mixing with water, other solvents, or other substances, their source and processing techniques, these raw materials either already contain a microbial load, or are vulnerable to subsequent microbial contamination, such that physical-chemical or preservative measures have to be considered for both their transport and storage. Due to the vast range of cosmetic raw materials, it is not possible here to list individual cosmetic ingredients. A comprehensive overview is provided by the “International Cosmetic Ingredient Dictionary and Handbook” (CTFA, 2002) and the “EU Inventory of Cosmetic Ingredients” (EU, 1996). These works classify ingredients commonly used in cosmetics, by INCI reference, chemical designation and synonym, chemical structure, cosmetic function and reference sources, and they form the basis of the declaration of contents required in many countries according to INCI nomenclature (International Nomenclature Cosmetic Ingredient). Table 6 gives an overview of the categorisation of the most important raw materials by function. Individual representatives of these raw material categories are discussed with respect to their microbial susceptibility and anti-microbial potential. While individual ingredients may have an impact on microbial growth – either inhibitory, or even lethal - no theoretical statement can be made of how they behave as a constituent of a complex formulation. Only appropriate testing can establish the anti-microbial efficacy of a formulation.

Water. Often water constitutes the largest single ingredient in a formulation and is extremely important as a primary source of contamination. For this reason, production water invariably undergoes some sort of

Table 6 General summary of cosmetic products based on Data by (Forsthoft, 1991)

Contents	Function
Water, alcohol	solvents or dispersion agents
Fats, oils, waxes	fat phase in emulsion, refatting agents in cleaning preparations
Surfactants	cleaning material, emulsifiers, dispersion agents
Glycols, sorbitol, glycerol	humectants
Thickeners, gelling agents	consistency agents
Skin conditioners	skin care
Special active substances	special care effects
Anti-oxidants, chelating agents, preservatives	product protection
Colourings	colour
Perfume oils, aromatic substances	fragrance

pre-treatment before use in the manufacturing process to reduce its micro-organism load. Various methods are used for this purpose, including UV irradiation, disinfection with ozone and its subsequent degradation by UV radiation, micro-filtration and treatment with low concentrations of hydrogen peroxide. With the latter technique, possible oxidative reactions in the end-product, resulting in alterations in colour or fragrance, have to be taken into account, although brief heat-shock treatment during production removes any residual hydrogen peroxide, avoiding any undesirable reactions later in production or the finished product. Discussion of the specialised technology required by all the above-mentioned techniques is beyond the scope of this chapter.

Alcohol. The anti-microbial efficacy of alcohol is universally acknowledged, and increases with increasing chain length. Propanol and isopropanol, although commonly used in the past and currently under environmental review, have been largely replaced by ethanol. The efficacy of ethanol depends on the concentration used and formulation composition. Products such as hair setting lotions or face lotions, that contain no other substance that supports microbial growth, can be preserved with as little as 10% ethanol. The use of re-fattening agents, or other microbe growth-supporting substances, require increased alcohol content. Products containing > 30% ethanol generally need no additional preservatives. Although ethanol exhibits good efficacy against bacteria and other microbes, it has little effect on bacterial spores and ethanolic raw materials and ingredients should be checked on delivery from the supplier for the absence of pathogenic sporogenous micro-organisms (see also, *Other additives*).

Fats, oils, waxes. Water-free fats, oils or waxes are generally not a source of microbial contamination, as they lack the biologically-available water necessary for the proliferation of micro-organisms. This group of compounds are typically used in emulsions or re-fattening agents in surfactant systems. Antioxidants, added to retard oxidation and prevent rancidity, can potentiate the efficacy of certain preservatives (5.9.4.3./*Antioxidants and chelating agents*).

Surfactants (Hill, 1995). Surfactants are surface-active compounds with various primary functions in cosmetic products. In shampoos, foams and shower-gels, syndets (synthetic detergents) etc., they act as washing and foaming agents. They are also used as emulsifiers, solubilizers or dispersion agents. Depending on chemical structure, concentration used and formulation pH, surfactants can exhibit anti-microbial efficacy. Surfactants can absorb and disrupt cell membranes, increasing their porosity. In addition to causing direct damage to micro-organism cell membranes, increased membrane porosity also facilitates the penetration of other anti-microbial substances.

Anionic surfactants. The most important representatives of anionic surfactants base on sulphates (fatty alcohol ether sulphates, fatty alcohol sulphates, alkylaminoethyl sulphates), sulphonates (sulphosuccinate, fatty acid isothionate, olefinic sulphonate) and on carboxylates (ethercarboxylate, acylglutamate and protein/fatty acid condensation products). Ethylcarbonic acids with 2.5 mol EO (lauryl alcohol ether carboxylic acid, sodium salt) exhibit good broad-spectrum anti-microbial efficacy, even at concentrations of 10–15%. All other compounds, unpreserved and used at concentrations < 20%, are particularly susceptible to micro-organisms. Fatty alcohol sulphate and fatty ethyl alcohol sulphates are the major anionic surfactants used in hair shampoos and hair cleansing products. (Kabara, 1984a) has described the anti-microbial efficacy of these anionic surfactants, although this is largely restricted to Gram-positive bacteria. Their action is most effective at acid pHs, although hydrolytic cleavage of the sulphate cannot be ruled out at pHs < 4.5. The susceptibility of Gram-negative bacteria to surfactants depends on their cell wall structure, which, to a lesser or greater degree, presents a strong diffusion barrier to surface active compounds. Combined with chelating agents, such as ethylenediaminetetracetic acid (EDTA), it is possible in some cases to penetrate this barrier (Hart, 1984). Below gelling point, diluted anionic surfactants (< 28%) are stabilized in strongly alkaline conditions (pH > 11) without additional chemical preservatives. In neutral or weakly alkaline environments, the addition of chemical preservatives is needed.

The monoglyceride derivatives of C₁₂ fatty acids are also anionic surfactants. Their anti-microbial efficacy is principally directed against Gram-positive bacteria. Shorter chain lengths (C₁₀–C₁₁) work mainly against yeast and moulds. The lack of efficacy cover against Gram-negative bacteria can be compensated by the use of anti-oxidants and chelating agents. Systems of 1–10% glycerol monolaurate [II, 9.8.]* are described as self-preserved and can be employed as emulsifiers in creams or deodorants (Kabara, 1984b).

Cationic surfactants. Cationic surfactants are known for their anti-microbial efficacy. However, depending on their structure, due to only moderate skin compatibility, and in some cases poor foaming properties, they are rarely used as the main cleaning agent in skin and hair cleansing products. Nevertheless, they are often used in low concentrations in hair conditioners and treatment preparations. They are also used in shampoos, to

* see Part Two – Microbicide Data

improve the ease of combing wet hair. Due to their anti-microbial action, certain cationic surfactants are listed as preservatives (EEC, 1976, Annex VI), including alkylamines (chain length C_{12-16}) [II, 18.2.], the quaternary ammonium compound group (QACs), alkylbenzyltrimethyl ammonium salts. QACs with good anti-microbial potential include the preservatives, cetyltrimonium bromide and chloride [II, 18.1.1.], benzethonium chloride [II, 18.1.3.], benzalkonium chloride, bromide, and saccharinate [II, 18.1.2.], which are most effective at neutral or alkaline pHs. Non-ionic surfactants, such as Polysorbate 80 can inactivate cationic surfactants, an effect that occurs above the critical micellization concentration [II, 18.], although below this point they have a potentiating effect (Schmolka, 1973).

Amphoteric surfactants [II, 18.4.]. The best known amphoteric surfactants include alkylamidobetain (e.g. cocoamidopropylbetain) and alkylamidoglycinate. Similar in structure to QACs, they also show anti-microbial efficacy (Garrand, 1985). In particular, a concentration of 15% has been shown to produce good anti-microbial efficacy. Amphoteric surfactants are frequently mixed with anionic surfactants to improve skin compatibility in hair and skin cleansing products.

Non-ionic surfactants. The addition of fatty acid monoethanolamides, fatty alcohol ethoxylates and alkylpolyglycosides (APG) improves skin compatibility and foaming properties of cleansing products, in particular APGs. However, these non-ionic surfactants provide no recognisable anti-microbial action.

Humectants. Sugars (e.g. sorbitol), glycerol and glycol (e.g. polyglycol, but also propylene, butylene and hexylenglycols) all bind water. At concentrations of 5–10% they can effectively reduce the amount of biologically-available water. Used at these high concentrations, they can contribute to product preservation, although in some preparations they can give rise to undesirable application effects, causing a 'sticky' sensation in creams, or poor foaming behaviour of a shampoo. These effects have to be considered when calculating an acceptable concentration of such substances. Glycols can be used to advantage, but should be accompanied by lipophilic preservatives, increasing the water solubility of latter and thus improving their bio-availability in the water phase.

Thickeners/gelling agents (Forsthooff, 1991). Fatty alcohol ethoxylate, fatty acid alkylolamine, ethoxylated glucose ester and common salt are used as thickening additives in liquid washing, shower and bath preparations. Structurally viscous products retain their gel-like character by the addition of polymer gelling agents such as, polyacrylic acids, xanthan or hydroxypropylmethylcellulose. However, the latter compound has been associated with a loss of efficacy for certain preservatives.

Emollients (Forsthooff, 1991). As a function of their chemical structure, emollients or skin conditioners undergo polar interactions and bind to the skin, producing a discernible smoothing of the skin's surface. Silicon derivatives, cation-active compounds, and more significantly, proteins, such as milk proteins and albumin hydrolysate are used as emollients, although the proteins are known to support the growth of micro-organisms.

Other additives. Plant extracts and mineral raw materials are variously used to produce additional care effects. Natural raw materials that micro-organisms can exploit need particular scrutiny. The major source of contamination found in these raw materials are spores, mycotoxins and clostridia. Microbial reduction measures, for example by irradiation sterilisation, is recommended before use. However, it should be borne in mind that although this treatment destroys the micro-organisms, the toxins they produce may still remain, and the threat of possible incompatibility problems cannot be ruled out. Furthermore, with the use of spore-contaminated raw materials, including those containing alcohol, the possibility of bacterial spores surviving a preservative system cannot be excluded. Even if such spores do not germinate in preservative systems, they remain viable during manufacture until conditions are suitable for germination, i.e., during consumer use. In some cases, low level concentrations of non-pathogenic spores are tolerable, but pathogen clostridium spores, for example, should be avoided at all costs: the prospect of such spores gaining access to an open wound, e.g. the application of a contaminated alcohol-based after-shave, is alarming to say the least.

In addition to primary-contaminated or microbiologically susceptible natural raw materials, many multi-active agents classes are based on an alcoholic or phenolic structure. These extracts, such as melissa extract, rosemary acids, phenylethylalcohol [II, 1.6.] etc., often show strong microbial efficacy and can be used to support product preservation in cosmetics (Eggensperger, 2001).

Antioxidants and chelating agents. Antioxidants inhibit the oxidative reactions in oils, fats and waxes that lead to rancidity, with its distinctively unpleasant smell. Due to their reducing properties, antioxidants only exhibit weak anti-microbial efficacy. However, they can enhance the efficacy of certain preservatives, such as Bronopol [II, 17.14.], Germall 115 [II, 3.4.7.], Glydant [II, 3.4.9.] and Kathon CG [II, 15.3.], allowing a reduction of the

Table 7 Preservatives potentiated by EDTA

Preservative	Synonym	Reference
2-Bromo-2-nitro-propan-1-ol	Bronopol	II, 17.14.
N-(3-chlorallyl)-hexaminium chloride	Quaternium-15	II, 3.3.2.
Imidazolidinyl urea	Germall 115	II, 3.4.7.
Dimethylol-dimethyl-hydantoin	DMDM hydantoin	II, 3.4.9.
2,4,4'-Trichloro-2'-hydroxy-diphenylether	Triclosan	II, 7.6.1.
2,4-Hexadienoic acid	Sorbic acid	II, 8.1.5.
o-Hydroxy-benzoic acid	Salicylic acid	II, 8.1.10.
p-Hydroxy-benzoates	Parabens	II, 8.1.11.
Cetyltrimethylammonium bromide (CTAB)	Cetrimonium bromide	II, 18.1.1.
N-Alkyl-N, N-dimethyl-N-benzylammonium chloride	Benzalkonium chloride	II, 18.1.2.
N, N-Dimethyl-N-2-2-4-(1,1,3,3-tetramethylbutyl)-phenoxy-ethoxy-ethyl-benzylammonium chloride	Benzethonium chloride	II, 18.1.3.

latter's concentration in preparations. 2-tert-butylhydroquinone (TBHQ), propylgallate (PG), butylhydroxyanisole (BHA) and butylhydroxytoluol (BHT) are acknowledged as effective antioxidants.

EDTA in its non-complexed form is known to exercise a synergetic effect on certain preservatives (Table 7). Due to differences in cell wall structure, chelating agents express positive synergy against Gram-negative bacteria, but not Gram-positive bacteria (Gilbert, 1988). For example, the presence of EDTA dramatically decreases the adaptive capacity of Gram-negative bacteria to QACs. Combined with other preservatives, EDTA increases efficacy such that concentrations of the former can be significantly reduced.

Perfume oils. The anti-microbial action of different fragrances and aromatics has long been known (Kabara, 1984b; Morris, 1979). Perfume oils are used to both mask the unpalatable odour of other raw material ingredients, and to increase the user's sense of well-being. However, many people react allergically to fragrances of all types: fragrances and perfumes head allergy 'hit' lists (Uter et al., 2001). The International Fragrance Association (IFRA) has set itself the task of publishing a positive list for the use of perfume-containing substances. Many anti-microbial fragrances and aromatic substances are of little practical value due to their relatively high skin incompatibility, and often unacceptably over-powering aroma or colour when used at concentrations likely to exhibit anti-microbial efficacy. In effect, few of this group of substances meet all the necessary requirements for a useful preservative agent.

Preservatives. Preservatives are generally understood to be chemical substances primarily used to preserve a product. Cosmetic preservatives must express low toxicity, good skin compatibility and protect the product from microbial adulteration. Within the EU, preservatives are regulated by annex VI of the Cosmetic Guidelines (76/768/EEC). In the USA, the FDA publishes a list of the commonly-used preservatives every two years. In addition to their function as a preservative, some of these compounds are used at higher concentrations to fulfil a special microbiological function. These compounds are listed in annex VI of the EU guidelines, marked with (+). Currently, discussions are underway to replace the (+) designation with an explicit description of specific substances. These preservatives target both specific micro-organisms and also contribute to the overall preservation of the product. The following substances fall into the category of "other uses".

Deodorants (Bremer, 1991). The most commonly used deodorant agents are Triclosan (2,4,4'-trichloro-2'-hydroxydiphenylether) [II, 7.6.1.], Chlorhexidin (1,1'-hexamethylenbis(5-(4-chloro-phenyl)-biguanide) [II, 18.3.4.] and TTC (3,4,4'-trichlorocarbanilide) [II, 10.8.].

However, in recent years use of these organo-halides has declined in Japan and Europe, and they are currently under environmental review. Many fragrance compounds used in deodorants have anti-microbial properties. Although naturally-occurring plant substances, many are synthetically produced and include Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol, terpenalcohol from lime tree leaf oil) and phenoxyethanol (naturally found in green tea). Subtle combination with various other active agents can increase the anti-microbial deodorant efficacy of these substances: e.g. a mixture of glycerol monolaurate [II, 9.8.], Farnesol and phenoxyethanol [II, 1.7.].

Anti-plaque additives. Chlorhexidine [II, 18.3.4.], Hexetidine [II, 3.3.26.], Triclosan [II, 7.6.1.] and sodium benzoate are [II, 8.1.9a.] the substances of choice (Kabara, 1984b).

Anti-dandruff agents. Zinc pyrithion [II, 13.1.3b.], piroctone olamine (1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2(1H)-pyridon-ethanolamine salt) [II, 13.1.2.] and salicylic acid [II, 8.1.10.] are the commonly used anti-dandruff additives.

Antiseptic substances. Alcohol, Triclosan [II, 7.6.1.], Trilocarban [II, 10.8.] and salicylic acid [II, 8.1.10.] are used as antiseptics (not classified as cosmetic substances in EU).

5.9.4.4 Selection of appropriate preservatives

When selecting preservatives or preservative systems for cosmetics, the single most important characteristic of the complete formulation is good skin compatibility. Preservatives react against micro-organisms, damaging or completely destroying the cell. However, preservatives also react with the cells of the skin. It is perhaps not surprising that increased skin intolerance to preservatives has been observed in recent years. Clearly the cosmetic chemist strives to ensure good skin compatibility and yet provide the product with an effective preservative system, a condition not always easy to fulfil. Orth, (1989) promotes the use of well-tolerated preservative combinations, particularly those in which synergetic effects permit significant reduction of the concentration of individual active ingredients, on the assumption that this reduces skin sensitisation and irritation. To minimize preservative levels Kabara (1984a) recommended exploiting the anti-microbial potential of the following materials: glycerol monolaurate, butylhydroxytoluol (BHT) and ethylenediaminetetraacetic acid (EDTA). Formulations with accredited self-preservative efficacy may well require no further addition of the chemical preservatives listed in annex VI of the EU Directive (76/768/EEC). However, some raw materials that display secondary anti-microbial efficacy can also cause skin irritation, a factor that requires consideration when planning their inclusion in a formulation. In addition to skin compatibility, a number of other factors have to be considered when selecting preservatives for effective formulation protection. Preservatives vary greatly in their individual characteristics, whether it is the efficacy spectrum, pH-dependent efficacy optimum, solubility, compatibility with other formulation ingredients, temperature and storage stability, or the compatibility with primary packaging materials.

Water solubility. Given that micro-organisms require water for growth, it follows that the water phase of a formulation requires protection against microbial spoilage. The water solubility of preservatives is an important factor when selecting preservatives. Often solubility can be improved by adding glycols or alcohols to the formulation. The alkali salts of organic acids and p-hydroxybenzoic acid esters are more readily soluble than the free acid or ester respectively.

Partition coefficient. Lipophilic preservatives tend to accumulate in the lipid phase of a formulation. This phenomenon is particularly important when planning preservative systems for emulsions, where the partition coefficient, as the ratio of preservative concentrations in the oil and water phases respectively, is a significant index of how they partition in emulsions. Adding alcohol to an aqueous milieu can shift the distribution coefficient in favour of the aqueous phase. In contrast, non-ionic surfactants tend to shift the partition coefficient in favour of the oil phase, resulting in a reduction of preservative efficacy in the water phase (Wallhäuber, 1984).

Efficacy spectrum. The efficacy spectrum of individual preservatives is often very different. Generally, one distinguishes between narrow and broad spectrum efficiency. Some preservatives work mainly against Gram-negative bacteria, whereas others are more effective against Gram-positive, and/or yeasts and moulds. A series of monographs document the effectiveness of individual preservatives against specific micro-organisms. The effective concentration against individual micro-organisms is either given as the minimum inhibitory concentration (MIC) or as minimum microbicidal concentration (MMC) in ppm or µg/ml. Minimum inhibition is understood as being the concentration required to inhibit growth. Similarly, the minimum microbicidal concentration is that at which the specified micro-organism is killed. These values are used to estimate the probable effective concentration of preservative and the constellation of preservatives needed to cover efficacy gaps.

pH value. pH is an important factor in any formulation, since individual preservatives express a wide range of pH optima. For instance, the efficacy of organic acids is expressed only by the undissociated fraction of the free acid [II, 8.], and pH > 5 are pointless. The antimicrobial efficacy of the great number of formaldehyde releasing compounds [II, 3.1. – 3.5.] and of PHB esters [II, 8.1.11.] is just so considerably dependent on pH. This is also valid for microbicides with activated halogen atoms [II, 17.] which are found among heterocyclic N,S-compounds [II, 15.], too. pH values > 8 cause cleavage of chlormethylisothiazolinone [II, 15.2.]; and subsequent inactivation is not a spontaneous process and only becomes apparent during storage. Other substances undergo similar degradation. Some of the most effective preservatives, i.e., chlormethylisothiazolinone / methylisothiazolone (3:1) [II, 15.3.] and dibromdicyanobutane [II, 17.18.] show good efficacy at pH values < 6, although they can be used up to pH 7.5. However, for sufficient preservative efficacy at these higher pHs, their concentration needs to be drastically increased. Other compounds work principally in alkaline milieu (e.g. Na-hydroxymethylglycinate [II, 3.5.1.] is effective at pH 8–12) and are ineffective in acid media. In the interests of good skin compatibility,

and to keep concentration as low as possible, careful consideration of preservative pH optimum is required when planning a formulation.

Temperature tolerance. The stability of some preservatives is also influenced by temperature. Although PHB esters [II, 8.1.11.] are, for example, relatively temperature stable and can be added to a recipe at 80°C, others such as isothiazolinone mixtures [II, 15.3.] and dibromodicyanobutane [II, 17.18.] can only be added to a formulation at temperatures below 40°C.

Tolerance of other formulation ingredients. Preservatives may well cross-react with other formulation ingredients. This includes all chemical reactions with cationic, anionic or non-ionic compounds, oxidising and reducing agents, and proteins etc.

Packaging material compatibility (Wallh      , 1984). The suitability of a preservative, depends not only on the breadth of its efficacy spectrum and the other criteria already mentioned, but also on primary packaging materials. Efficacy reduction in this case is mainly due to absorption effects, and depends on the type of formulation – how lipophilic the preservatives are and the plastics used. Plastics such as polyethylene absorb quaternary compounds [II, 18.1.], PHB ester [II, 8.1.11.], sorbic acid [II, 8.1.5.] benzoic acid [II, 8.1.9.] etc., from fluid and semi-fluid contents, leading to a reduction in their efficacy, often within weeks.

On the other hand, packaging plastics should not release any substances into the formulation (e.g. softener) that might inactivate anti-microbial substances.

Surface effect. In some cases, microbial growth is observed on the surface of the product in a container, although the rest of the product shows no signs of microbial activity. Such an effect can be triggered by a decrease in preservative concentration due to dilution by a film of water condensation on the product surface. However, Eigener (1995) claimed such surface contamination to be a temperature-independent effect and demonstrated that even moulds inoculated into the product and subsequently destroyed by the product's preservative system were, however, able to grow trouble-free on the product surface. Contamination was shown to be due to single air-borne spores or contaminants on the packaging materials. The penetration of preservatives into the surface layer has been described as problematic, since spore seeding and their subsequent germination may be possible. In such cases, mould growth has been observed on a thin product film coating the container walls above the product surface layer, or directly on the surface layer itself. In such cases, the use of a vapour effective preservative, such as formaldehyde might make sense.

Preservatives with broad spectrum efficacy. Organic acids [II, 8.1.]. Organic acids such as benzoic acid, salicylic acid, sorbic acid, dehydroacetic acid and formic acid are widely used as preservatives in skin and hair cleansing preparations. The efficiency of organic acids is limited by pH. As only the undissociated fraction of the acid exhibits antimicrobial effectiveness, pH should not exceed pH 5.0. Efficacy declines dramatically at higher pHs. Table 8 lists the percentage fraction of undissociated acid related to pH. Inaccurate ingredient dosing during production can strongly influence the efficacy of organic acids. For instance, permissible gravimetric or volumetric tolerances for ingredient dosing range from ± 5 to 10%. A tolerance range of ± 0.5 pH units is realistic when measuring pH and organic acid preservative concentration often ranges from 0.1–0.5%. In practice, strict attention should be paid to these tolerances and the conformity of product pH with the pH optima of the preservatives. The poor water-solubility of the free acid favours the use of the more readily soluble alkaline salts which, however, may increase the pH of the formulation, and perhaps necessitate pH corrections.

Dibromodicyanobutane (DBDCB) [II, 17.18.]. Due to its poor solubility in water, this microbicide is often supplied as a mixture with 2-phenoxyethanol. DBDCB presents an excellent preservative, possessing good efficacy against bacteria, yeast and moulds. It is fully effective in an acidic environment and efficacy extends up to pH 8, although falls off significantly at pHs > 6 . Reduced efficacy can also occur if used in highly pigmented cosmetic formulations. In such cases, this short-fall can be compensated by either increasing the concentration of DBDCB, or supplementing it with another preservative. Combination with PHB esters [II, 8.1.11.] and

Table 8 Percentage of the undissociated form of organic acid preservatives

Preservative	pH5	pH6	pH7
Benzoic acid	13	1.5	0.15
Propionic acid	42	6.7	0.71
Salicylic acid	0.94	0.094	0.0094
Sorbic acid	37	6.0	0.6
Dehydroacetic acid	65	15.8	1.9

phenoxyethanol [II, 1.7.] demonstrates particularly good efficacy. Di-bromdicyanobutane is inactivated by reducing agents, and working temperatures should not exceed 40°C.

Isothiazolinones [II, 15.3.]. Another agent with excellent anti-microbial properties, with excellent efficacy against bacteria, yeasts and moulds, is an isothiazolinone-based product, consisting of a 3:1 mixture of chloro-methylisothiazolinone (CMI) and methylisothiazolinone (MI). This mixture, which is used with a maximum concentration of 15 ppm A.I (active ingredient), is, however, inactivated by strong oxidizing and reducing agents and should not be processed at temperatures higher than 40°C. As already mentioned, pH values >8 lead to denaturation of CMI. Efficiency starts to fall off at pH 6. Synergistic effects have been ascribed to mixtures with DBDCB in the presence of formaldehyde-liberating substances. As a result of increased sensitization, associated in particular with CMI, preservatives containing CMI/MI are currently primarily used in rinse-off rather than leave-on products.

Substances with limited efficacy. Alcohols. As mentioned earlier, ethanol [II, 1.1.], phenoxyethanol [II, 1.7.], benzyl alcohol [II, 1.4.] and dichlorobenzyl alcohol [II, 1.5.] can be used as preservatives.

Phenoxyethanol [II, 1.7.]. Phenoxyethanol is not only used as a preservative but as an anti-microbial agent in deodorant formulations, too. The usual concentration ranges from 0.5 to 1.0%. Used alone in cosmetic formulations, its efficacy is rarely sufficient. Phenoxyethanol is more effective against Gram-positive bacteria than Gram-negative bacteria. To plug this gap in efficacy, other preservatives such as dibromodicyanobutane [II, 17.18.], PHB ester [II, 8.1.11.], iodopropinylbutylcarbamate (IPBC) [II, 11.1.], imidazolidinyl urea [II, 3.4.7.] and formaldehyde [II, 2.1.] are used. Optimum pH is 4 to 5.

Benzyl alcohol [II, 1.4.]. In the EU, benzylalcohol's anti-bacterial efficacy can be exploited up to a concentration of 1%. It is used at higher concentrations in hair colours, but chiefly as a solvent. Benzyl alcohol is effective against Gram-positive bacteria. Efficacy against Gram-negative bacteria, yeasts or moulds, often requires higher concentrations than the permitted 1%. Optimum efficacy is at pH 5–6. Atmospheric oxygen can cause slow oxidation of benzyl alcohol to benzaldehyde, resulting in a strong bitter almonds smell.

Dichlorobenzyl alcohol [II, 1.5.]. This compound is effective in a pH range of 4–10 and can be used as a stabilizer for alkaline products. Dichlorobenzyl alcohol exhibits good efficacy against a wide range of bacteria, yeasts and moulds. However, this preservative is poorly water soluble, but dissolves well in propylene glycol. Where preservative efficacy is inadequate, it can be used in conjunction with Bronopol [II, 17.14.], Parabens [II, 8.1.11.], isothiazolinones [II, 15.3.] or Germall [II, 3.4.7.].

Formaldehyde [II, 2.1.] and *formaldehyde-releasing compounds* [II, 3.1.–3.6.]. Formaldehyde is effective against bacteria and moulds, although reactions with proteins can reduce efficacy against the latter, and in such cases it should be supplemented with a substance effective against fungi such as Parabens [II, 8.1.11.] or IPBC [II, 11.1.]. Formaldehyde tends to polymerize at pHs around 6–8. Notwithstanding topical discussions of sensibilization risk and other possible disadvantages associated with formaldehyde are not valid for legally-proscribed concentrations used in cosmetics which represent no such risks. Formaldehyde-releasing substances have the advantage that they do not liberate their formaldehyde content all at once. The urea derivative, imidazolidinyl urea [II, 3.4.7.] is used at concentrations between 0.2 to 0.4%. To cover an efficacy gap against moulds, Parabens are often used. 1,3-dimethylol-5,5 dimethylhydantoin (DMDM-hydantoin) [II, 3.4.9.] dissolves relatively well in the water, is compatible with proteins and can be used over a wide range of pHs, but is also less effective against moulds, and requires combination with an appropriate fungicidal agent. Similarly, N-(3-chloroallyl)-hexaminiumchlorid (Dowicil 200) [II, 3.3.2.] exhibits poor efficacy against moulds and requires the appropriate combination with additional fungi-effective substances. It is used at concentrations of 0.1–0.2%, in a pH range of 4–10.

In cosmetics, use of 5-bromo-5-nitro-1,3 dioxan (Bronidox) [II, 17.15.] and 2-bromo-2-nitro-propane-1,3-diol (Bronopol) [II, 17.14.] has declined dramatically, as there exists the risk that in cosmetic formulations containing amines N-nitroso compounds may originate which are known as carcinogens.

In the EU these substances therefore may only be used as cosmetic preservatives if nitrosamine production is prevented.

Quaternary ammonium compounds [II, 18.1.]. As these compounds show poor compatibility with anionic and non-ionic surfactants, they are rarely used as cosmetic preservatives. They are more effective in alkaline than in acidic environments, display poor efficacy against pseudomonads, but are highly effective against other Gram-negative bacteria, Gram-positive bacteria and moulds. Cetyltrimethylammonium bromide and benzalkonium chloride are listed as preservatives in EU guidelines.

Other cationic agents (Eigener, 1995). Due to their cationic nature, Chlorhexidine [II, 18.3.4.] and polyhexamethylenbiguanide [II, 18.3.3.] are easily deactivated by anionic surfactants and many polymer compounds, which somewhat limits their scope for use. The addition rates move between 0.05 and 0.2%. At sufficient concentrations, these compounds exhibit good antimicrobial efficacy at pH 5–7.

Phenol derivatives. o-phenylphenol [II, 7.4.1.], p-chloro-m-cresol [II, 7.3.1.] and isopropylcresol [II, 7.2.2.] are intended for use in alkaline environments and are therefore seldom used in cosmetics. All phenols are inactivated by inclusion in micelles which detergents/surfactants (non-ionic and anionic) form in solutions as soon as their concentration exceeds the critical micellization concentration (see Part II, 7. and 18.).

2,4,4'-trichloro-2'-hydroxy-diphenyl ether – Triclosan [II, 7.6.1.]), (DGK, 1995). Triclosan exhibits good bactericidal efficacy, but is more effective against Gram-positive than Gram-negative bacteria. In particular, it is poorly effective against pseudomonads, but shows good efficacy against much of the human axilla's bacterial flora (staphylococci, coryne-bacteria) and *Actinomyces* and other anaerobe micro-organisms inhabiting the oral cavity. Triclosan is effective between pH 4 to 8, with an optimum at pH 5. At pH 8 only bacteriostatic efficacy is demonstrable. Triclosan's efficacy spectrum is generally insufficient for use as a preservative, however it does find 'other uses', e.g. in deodorants, antiperspirants in aerosol and non-aerosol form (stick, rollers), liquid and solid toilet soaps, syndets (deodorant soaps), medical soaps, creams, lotions, salves, dental care products and mouthwashes.

Parahydroxybenzoic acid esters (Parabens) [II, 8.1.11.]. Parabens are frequently used as preservatives. Esters of p-hydroxybenzoic acid (PHB) are generally more effective against yeast and moulds than bacteria, and in turn against Gram-positive bacteria than Gram-negative bacteria. Kabara (1984a) described an increase in efficacy if PHB esters are supplemented with EDTA. Optimum pH lies between 4 and 8. Antimicrobial efficacy increases with increasing chain length, although water solubility correspondingly decreases. In practice, short-chain compounds such as the methyl, ethyl and propyl esters are preferred. The concentrations used for the methyl ester are 0.15%–0.3%, ethyl ester 0.05–0.2% and propyl ester 0.02–0.04%. Mixtures of higher esters in glycol are available as formulated preservatives (e.g. Phenonip). Non-ionic surfactants can inactivate PHB esters (micellization). Due to their lipophilic character, PHB esters tend to accumulate in the oil phase of emulsions. The availability of water is markedly improved by adding propylene glycol or other higher poly alcohols. It is also customary to combine the esters with phenoxyethanol. This often provides sufficient efficacy cover for formulations susceptible to moulds.

3-iodo-2-propinyl-butylcarbamate (IPBC) [II, 11.1.]. IPBC is a good alternative to PHB esters. This compound is also preferentially effective against yeast and moulds. Good efficacy is achieved with concentrations as low as 0.005–0.01%. However, IPBC is poorly soluble in water and is preferentially pre-dissolved in glycols. As with PHB esters, efficacy and water availability are significantly increased by the addition of phenoxyethanol. This preservative is used at pHs from 4–10. In the EU the agent may not be used as a preservative in either lip care products and lip cosmetics, or oral hygiene formulations. 0.05 % IPBC are permitted for the preservation of other cosmetic products with the limitation that "leave on" products containing > 0.02 % IPBC have to be labelled "contains iodine".

5.9.5 Validation of effective preservation

Adequate preservation is said to guarantee effective protection if, during storage and product use, both as instructed or in any other foreseeable manner, it suffers no spoilage, or the growth of undesirable, or pathogenic organisms.

To fulfil these requirements, not only the selection of preservative type and concentration needs careful consideration during formulation development, but also the type and extent of potential microbial influences which might adversely effect the quality of the finished product quality. The microbial quality of raw materials is a particularly important factor, but the provision of comprehensive production instructions, covering the processing of preservatives and plant hygiene, from the receipt of raw materials to the dispatch of the end product is also vital.

5.9.5.1 Types of primary packaging

The type of primary packaging also has an influence on product protection during use by the consumer. Aerosols generally provide good product protection. Filled into the can and pressurized with propellant gas, the very nature of its packaging protects the product from exposure to potential contamination. Nevertheless, even this

apparently secure packaging can pose a microbial risk if, before charging the can with propellant gas, the product is already contaminated with bacteria or moulds. Aerobic bacteria deprived of oxygen would not survive long, but problems could arise from the toxins they produce. Furthermore, some studies have shown that moulds can survive in today's CO₂ or propane/butane-charged aerosol cans. Therefore, preservative protection should also be planned for products filled in aerosols. The demands on microbiological stability during storage and consumer use are considerably less for single-use products, as the exposure to potential contamination during use is negligible: the product need only be filled microbe-free in its packaging. Pump systems, tubes and containers with narrow openings also represent excellent product protection design during use. The risk of contaminated shower or bath water gaining access to a shampoo or shower gel during use is greatly diminished by equipping its container with a narrow opening. Cosmetic products packaged in wide mouthed jars probably present one of the greatest challenges for any preservation system, with their large surface area exposed to a moist, microbe-contaminated environment, and repeated contact with the contaminated hand of the user. Re-fill systems can also support the growth of micro-organisms. Work by Heinzel (1999a) on re-fill systems for hand cleansing materials in a dispenser system showed that dispenser construction and the preservatives used have an influence on the microbiological quality of a product.

The above-mentioned microbiological degree of influence must also be considered in its completeness and can vary from product to product. A product physically protected against contamination generally requires less preservation, than one exposed to a wide range of potential contamination sources during use, such as the large surface area of a repeated-use container.

5.9.5.2 Preservative challenge test (Heinzel, 1999b)

The efficiency of a preservative system cannot be estimated by theoretical consideration of formulation type and ingredients. Numerous factors influence the microbiological quality of a complete formulation. Even relatively minor alterations to a well-established formulation (colour or fragrance modifications) already equipped with a proven preservative system, require re-verification of adequate protection. Given that good pre-preservation of a product's ingredients contributes to its overall stability, it is hardly surprising that changes in supplier and raw material quality can influence the biological activity of the system. Experience has shown that changing the type of manufacturing process (i.e., machine and equipment changes, switching from vessel to continuous flow dosing manufacture) can have a powerful influence on the bio-availability of preservatives in emulsions. Even a quantitative assay of preservatives in the end product does not necessarily provide assurance of sufficient preservative cover. Furthermore, variations in production water can affect product protection. Since shampoos, shower and foam bath gels are generally uninfluenced by electrolytes, they are manufactured with de-ionized or mains water. Even if care is taken to ensure that the mains water used is microbe-poor, other constituents, such as divalent alkaline earth ions can influence microbial stability: some organisms, such as *Pseudomonas putida* in particular, can stabilize in the presence of Ca²⁺ and Mg²⁺. For this reason, production water needs to be checked by trials during formulation development phase.

The effectiveness of product preservation should either be validated by challenge or in-use tests, preferably a combination of both. The principle of preservative challenge tests is based on artificial contamination of a product with various test bacteria, yeast and moulds. Their subsequent growth behaviour is noted and evaluated over a defined time period. Challenge tests attempt to model the response of a product's preservative system to the micro-organisms it is likely to encounter during its manufacture and use, with a safety margin of multiple log₁₀- levels. However, exact simulation is unrealistic and the results of challenge tests should only be viewed as prognostic. Nevertheless, experience has shown that user tests cannot replace preservative challenge tests, as they are principally intended to determine the appropriate combination of preservatives and their concentrations. In the interests of maintaining good skin compatibility, the concentration of preservatives used should be kept as low as possible, with the added advantage of keeping costs down. However, under dosing preservatives might encourage micro-organism adaptation to the system (5.9.3.2. Product contamination) and result in spoilage of the product.

Most countries currently lack legally-binding regulations for the evaluation of preservative tests in cosmetics. Although manufacturers can in practice decide for themselves how they carry out and evaluate challenge tests, they are encouraged to follow guidelines and standards published in the appropriate pharmacopoeias: British Pharmacopoeia (BP, 1980); European Pharmacopoeia (EP, Council of Europe, 1997); United States Pharmacopoeia (USP, 1995). Evaluation criteria outlined in the pharmacopoeia are not, in practice, sufficient to guarantee the preservative stability of products marketed in many of the common repeated-use containers. Even the CTFA microbiology guidelines (CTFA, 2001), although giving detailed recommendations for carrying out preservation challenge tests, leave interpretation and evaluation of the results to the manufacturer's discretion. The CTPA (1990) currently recommends adoption of BP evaluation criteria. The acceptance criteria for cosmetics reprinted in Table 9 are set too low for products packaged in wide-mouthed repeated-use containers, with their large open surface area exposed to environmental influences. The evaluation of preservative challenge tests must include due

Table 9 Acceptance criteria for challenge tests

	Initial micro-organisms count	Reduction rate	
USP	Bacteria 10^5 /g	3 log ₁₀ within 14d	no increase up to 28d
	Fungi 10^3 /g		no increase up to 28d
CTFA	Bacteria 10^6 /g	3 log ₁₀ within 7d	no increase up to 28d
	Fungi 10^4 /g	1 log ₁₀ within 7d	no increase up to 28d
BP (topica)	Bacteria 10^6 /g	3 log ₁₀ within 2d	reductin to < 10 in 7d
	Fungi 10^6 /g	2 log ₁₀ within 7d	reduction to < 10 in 28d

consideration of product-specific risk potential, and can not simply be applied in a blanket fashion. In preservation challenge tests, the fate of the inoculated micro-organism is observed and noted over a defined time period: whether growth is inhibited or the micro-organism is killed. Depending on a product's risk potential, mere inhibition of the micro-organism may not in every case necessarily guarantee adequate preservation.

Evaluation of a challenge test is related to the stability of a formulation during manufacture, storage and its use by the consumer. It is recommended that all these aspects are given due weight when carrying out such tests by undertaking the following:

1. Validation of preservative efficacy in freshly-prepared laboratory material.
2. Validation of preservative efficacy after storage in end-container, to reveal possible interference with packaging materials. Product storage time and temperature during the test phase are manufacturer-specific, and validation tests should be adapted accordingly. They should also mirror conditions prevalent in extreme user environments, for example in the tropics. In each case, however, concurrence with national regulations concerning 'best before date' is obligatory. Cosmetic manufacturers in the EU are obliged to ensure a 'best before date' of at least 30 months (RL EC/76/768), in the absence of any other date indicated on the packaging.*
3. Validation of preservative efficacy in the first production batch just prior to packaging, thereby revealing all possible influences arising during the entire manufacturing process.

To evaluate the microbiological quality of a product, the results of challenge tests are collated and a prognosis produced. Further information on product microbiological stability is obtained from long-term observation of the product in the market. Long-term *in vitro* tests must show good reproducibility, and this can only be achieved if all test parameters are adequately standardized. The following section outlines the most important aspects of the challenge test design.

The Inoculum. Standardized test strains of specified taxa are recommended for use in preservative challenge tests. These can be obtained from official cell culture collections, such as the ATCC (American Type Culture Collection), and have the advantage of possible access to organisms of the same identity, even when switching strain. In addition, contaminants that occur in practice, such as those encountered during production or in end-products, can and should be used in challenge tests. Strains from this source generally involve micro-organisms that have adapted to the preservative system and demonstrate a higher resistance behaviour compared to culture collection strains of the same type. To ensure the retention of strain-specific characteristics, it is important that these strains are maintained on their original substrate, since cultivation on conventional nutrient media invariably causes them to lose their resistance potential, often after the first passage. However, cultivation of such 'in-house' strains has the disadvantage that with longer storage there is always the risk of additional product colonisation by other contaminants and even loss of the strain should the substrate be exhausted. Preservative challenge tests should not rely solely on the use of 'in-house' test strains, as it makes sense to always have recourse to standard test cultures.

Strain maintenance is an important component of any standardization protocol, and involves the standardization of strain storage and culture conditions (time and temperature) and nutrient medium selected. Whether strains should be cultured in fluid or on solid media, and frequency of sub-culturing are additional factors that require due consideration.

Common test cultures. The most common test strains, described in various pharmacopoeia test procedures, are the potentially pathogen representatives of Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), mould (*Aspergillus niger*) and yeast (*Candida albicans*). For cosmetic testing this range needs expanding to include strains largely responsible for the contamination and spoilage of cosmetics, e.g.: *Enterococcus faecium*; *Enterobacteriaceae*, such as *Enterobacter gergoviae*, *Proteus spp.*, *Klebsiella spp.*; non-fermenters, such as *Burkholderia cepacia*, *Pseudomonas putida*, *Pseudomonas fluorescens*;

* For such products, there shall be also an indication of the period of time after opening for which the product can be used without any harm to the consumer (7th Amendment EC/76/768).

moulds, *Penicillium spp.*, *Fusarium spp* and *Trichoderma spp.*; and yeasts such as *Candida parapsilosis*. Spore-forming bacteria, such as *Bacillus*, and less often, *Clostridium*, are also known microbial contaminants of cosmetics. However, the use of these micro-organisms in challenge testing presents some difficulties. In their vegetative form they are relatively easily killed and rarely merit specific inclusion in the challenge test strain profile. Their sporulated form is more significant, although preservative systems generally inhibit germination, they may not necessarily kill bacterial spores. This in turn can cause problems when evaluating preservative challenge tests, and as such they are rarely used as test strains.

Product samples can be inoculated with each strain administered separately. Alternatively, samples can be given as bacterial or fungal 'cocktails', as a single shot. Although the use of bacterial or fungal mixtures offers dramatic savings in time and cost, one must be sure that the micro-organisms selected exercise no mutually detrimental influence on growth, and that any special nutrient requirements are met by the test product. One disadvantage of using micro-organisms mixtures is that monitoring the growth kinetics of individual strains is not possible. Total evaluation is possible at the end of a defined test period.

Inoculation volumes. Inoculum volume should not exceed 1% of that of the test product sample, to avoid altering the latter's physical and chemical properties. Contaminants collected from real-life instances of product contamination should remain in their substrate, and be inoculated directly into the test product sample at not more than 10% of the latter's volume. To ensure good reproducibility, the inoculum and test product sample must be well mixed, preferably with a glass rod or stirrer. Use of an Ultraturax should be avoided, as the very high shear forces involved can damage the test micro-organisms. Poorly homogenized test samples may lead to localised microbial growth, so-called 'nesting'.

Incubation. The inoculated test samples can be stored at room temperature, or at even higher temperatures intended to simulate specific environmental conditions. Temperatures between 20–40°C support the growth of micro-organisms and their, possible, reaction with active ingredients of the preservatives leading to biodetoxification of a. i.'s. Aliquots of the artificially-contaminated product are sampled at specified intervals over a period of 28 to 35 days for micro-organism content.

Determination of micro-organism content. To estimate the micro-organism content of a sampled aliquot requires selecting subculture conditions (medium, temperature, dilution, incubation temperature and time) appropriate for unrestricted growth of the preservative-stressed micro-organisms, and adequate inactivation of the preservative system carried over in the sample aliquot.

Inactivation. Inactivation is considered successful if the recovery rate of the inoculated micro-organisms at time zero deviates no more than 1 log₁₀ level from the theoretically expected number of micro-organisms. Survival rate can be either qualitatively or quantitatively assessed. With the quantitative assessment method, the kinetics of lethality can be used to calculate the D-value (see D-value). However, both assessment methods require proof of adequate preservative inactivation. Experience shows that some preservatives, e.g. chlorhexidine and high concentrations of QACs, are relatively difficult to inactivate, a factor to which Gram-positive bacteria are notably more sensitive than Gram-negative bacteria. Irrespective of whether a tried and tested inactivation protocol is routinely used, a test of adequate inactivation must be carried out. Any post-active preservative effect which inhibits or prevents the growth of the micro-organisms present on such culture media can lead to false negative results. Rinsing solutions typically contain the following inactivating substances: 3.0% Tween 80, 0.3% lecithin, 0.1% histidine and 0.5% sodium thiosulphate or cysteine. Successful inactivation is indicated in non-growing sub-culture of the test product after renewed inoculation with a test strain (e.g. *Staphylococcus aureus*). Only when this test gives rise to growth and the concurrently run product-free culture shows similar growth, the product subculture is considered sufficiently inactivated.

D value. Under certain conditions a challenge test can be accelerated, whereby the D-value of living micro-organisms is extrapolated for the first 24 hours. The D-value is understood as the time required to effect a reduction in microbe number by 1 log₁₀ level from the theoretical value. With the help of this method, it is possible to estimate when the acceptance criteria are likely to be met, without running the test to this time point. To retain statistical robustness, this apparent gain of time, however, is only bought at the cost of undertaking 10 parallel test runs to calculate the D-value. To determine the D-value the following conditions must be met:

1. Single strain challenge
2. Quantitative determination of viable germ count
3. Preservation must reduce microbe numbers by several orders of magnitude in the first 24 hours
4. The death curve must fit a linear regression
5. Sufficient data is gained at the first reading point to generate a regression.

The reliability of these tests is limited by the speed of growth of the test organism. Cases where micro-organisms only show rapid growth after 2–3 weeks (e.g., behaviour observed in the case of *Burkholderia cepacia*) can not be assessed by this method.

5.9.5.3 *In-use tests*

As previously mentioned, the results of a preservation challenge test merely represent a prognosis of the expected product stability. For formulations subject to minor alteration this *in vitro* test is generally a sufficient confirmation of product stability. The results of preservative challenge tests for new formulations invariably require confirmation of product stability under conditions of practical use. Such in-use tests are time-consuming and cannot always be planned into the development phase. Normally, they are carried out by testing a minimum of 50 product samples in a home-use scenario (Heinzel, 1999b). Product samples known to be microbe-free are delivered to test households varying in the number of occupants and frequency of product use, thus ensuring a wide range of potential microbial loading during use. Users are instructed in the use of the product but are not informed of the reasons for the test to avoid adversely influencing normal use behaviour. The product is handed over for analysis immediately when approximately $\frac{3}{4}$ has been used. For meaningful evaluation of product stability it is important that microbe number is estimated as closely as possible to the time of last use. The following parameters are evaluated: noticeable changes in appearance and smell, total number of aerobic micro-organisms, number of spore-forming bacteria, and number of yeast and fungi.

As with evaluation of preservative challenge tests, manufacturers are at liberty to set their own evaluation criteria for such tests. When evaluating user-tests, it is reasonable to expect the presence of micro-organisms in the product immediately after use. Analogous to the preservation challenge test one would expect a reduction in microbe number over a pre-specified time. Returned product samples with $> 10^3$ cfu/g suggest product preservation is insufficient. Lower microbe numbers should be reduced over an acceptable time period to ≤ 100 cfu/g.

5.9.6 Production hygiene (Scholtyssek, 1999)

After successful completion of the development phase, the formulation is up-rated to production dimensions, a major component of which is the successful transfer of microbiological quality validated in the laboratory to the production process. Challenge to the preservative efficacy of a product should be avoided at every stage of manufacture. Even slight contamination, usually dealt with by the preservation system during subsequent production phases, should be avoided, as it may lead to reduced preservative efficacy during storage and limit stability during consumer use.

To ensure good product quality, COLIPA (CGMP, 1994) and the Council of Europe (GMPC, 1995) issued guidelines for good manufacturing practice for cosmetics, recommended as state-of-the-art standard technology for all cosmetic manufacturers. GMPC/CGMP recommendations however provide only rather broad guidelines and leave cosmetics manufacturers scope to develop their own practical solutions to production hygiene. For the production of their products, the cosmetics manufacturer needs to make provision for suitable space, low-microbe ventilation, technically adequate equipment and well-trained personnel. Materials flow and production processes should be created and codified as a set of production instructions.

Raw materials. An essential condition for the manufacture of cosmetics is the use of raw materials containing the lowest possible level of microbes. Microbiological susceptibility of individual raw materials determines which raw materials need to undergo routine microbiological testing on receipt from the supplier. When negotiating with suppliers of raw materials prone to microbial contamination, the manufacturer should require that the supplier provide microbial specifications for their goods, which can be used as a reference check for subsequent deliveries. Raw materials stored in tanks require particular vigilance. It is common practice to pump a new delivery of raw material directly into a storage tank that often contains the remains of previous deliveries. To avoid possible contamination of the new delivery, such remains should first be tested for microbe content. The salient priority of raw material quality checks is the monitoring of production water, as it often constitutes the greatest part of a formulation. Options for reducing the viable germ count of production water have already been described in section 5.9.4.3., and it should be subject to routine and rigorous quality checks.

Effective cleaning and disinfection. A further essential pre-requisite for the production of cosmetic products is the ease with which production and ancillary equipment can be cleaned. As a rule of thumb, a production line which does not automatically empty its entire contents and, due its construction, cannot be purged *in situ*, has to be considered a potential source of contamination. Effective cleaning and disinfection procedures require that the plant itself is cleanable. Only production plants constructed in compliance with rules for hygiene technology (EN 1672-2) may be cleaned automatically according to Cleaning In Place procedure, without recourse to

Table 10 Spectrum of disinfectants activities

Type	Halogens	Peroxides	Aldehydes	Alcohols	Phenolics	QAC Biguanides
	II.21.2.*	II.21.1.*	II.2.*	II.1.*	II.7.*	II.18.*
Gram (-) bacteria						
Gram (+) bacteria						
Myco- bacteria						
Bacterial spores						
Yeasts						
Moulds						
Viruses						
Technical properties	Protein sensitivity risks of corrosion		Smell	Inflammable	Smell	Adsorption effects
		good efficiency		limited efficiency		not efficient

*substances described in Part II-Microbicide data

disassemble. All other plants and components have to be dismantled and manually cleaned, using alkaline or acid cleaning agents, depending on the type of soiling involved. Disinfection is only carried out after thorough rinsing with microbe-sparse water. The common biocides used for disinfection are listed in table 10. To ensure the effectiveness of cleaning and disinfection procedures, these procedures need to be validated for each application.

Preventing cross-contamination. The co-transport of micro-organisms must be prevented when transporting raw materials, packaging materials and products out of the 'unclean zone' to the 'clean zone'. To avoid such cross-contamination, appropriate quality assurance measures should be in force along the entire transport route through the factory. Every stage of the manufacturing process should be subject to analysis for potential sources and routes of contamination, and when necessary, operational-specific instructions created and put into practice. Copies of these instructions should be distributed proactively to production staff in the form of training sessions. Table 11 lists basic guidelines for a systematic approach to securing production processes against the risk of contamination. An essential part of CGMP/GMPC compliant manufacture requires that all procedures created and enforced be subject to comprehensive documentation.

5.9.7 Fault prevention analyses (Scholtyssek, 1999)

Adherence to GMP guidelines already offers a sound basis for avoiding microbial contamination or other products mishap. However, in some cases it is also wise to carry out special manufacturing process fault prevention analyses. Similar approaches have already proven their worth in other industrial sectors, such as automobile and foodstuffs. The conceptual basis for such studies are FMEA, Failure Mode and Effects Analysis-Fehlermöglichkeits- und -Einflussanalyse (DIN 25 448), and the HACCP concept (Hazard Analysis Critical Control Points, (Pierson, 1993).

Fault prevention analyses can yield valuable information about processes of which the cosmetic industry has little experience. They can also be used to effect optimization of processes that repeatedly yield unexplained contamination. Fault prevention analyses, as applied to the cosmetics industry, are not only concerned with health aspects of cosmetics manufacture, but include exploration of faults that could incur economic losses and damage to company image.

The analysis is carried out by an inter-disciplinary team, generally recruited from product development, engineering, storage, manufacture, filling, quality assurance and microbiology. According to demand and problem definition, representatives from other disciplines can be co-opted onto the team.

Analyses conducted according to HACCP always only deal with a specific product and specific process steps. The focal point of these analyses is the determination of potentially dangerous microbiological risks and the so-called critical control points (CCPs). By definition CCP are process steps or phases in which an acknowledged risk can either be eradicated by target-controlled measures, or at least reduced to an acceptable level.

Table 11 Practical hygiene recommendations according to Cosmetic GMP guidelines. (based on data from Scholtyssek, 1999)

Examples for hygiene measures in cosmetic production processes according to CGMP guidelines
1. Working up of hygiene measures for storage, manufacture and filling areas 2. Drafting rules for personnel behaviour, specified for each production sector 3. Drafting hygiene measures for the handling of raw materials – Instructions for handling the incoming material and the storage of goods in sacks and drums – Measures for delivery and discharge of microbiologically-susceptible raw materials in tanks. – Construction and treatment of tanks, drafting a standard operation procedure for cleaning and disinfection, including cleaning frequency, the cleaning agents used, and their concentrations and reaction times. – Hygiene measures for the weighing procedure of raw materials, hygienic treatment for equipment, correct labelling of the containers, procedures for the handling of incompletely used open containers, bags, drums, etc. 4. Measures and controls for surveillance of production water. Controls (physical and microbiological) for monitoring the efficacy of the microbe germ reduction system used, and monitoring the quality of water used in production and filling areas, as well as in cleaning zones. 5. Production Area – Setting cleaning and disinfection intervals for equipment used for production and intermediary storage of bulk products. – Establishing standard operating procedures, with instructions for execution, materials to be used, their concentrations and duration. – Rules for personnel behaviour when handling microbiologically-susceptible premixes and bulk products. – Setting maximum standard times for microbiologically-susceptible premixes and bulk products. 6. Filling Area – Setting cleaning and disinfection intervals for the filling machines. – Establishing standard operating procedures, with instructions for execution, materials to be used, their concentrations and duration. – Hygiene measures for handling packing materials – Hygiene measures for handling end-product residues/bulk returns/corrections. 7. Measures for the microbiological release of products.

Focus tends to be directed towards physical or chemical control parameters, such as pH, temperature, etc., rather than microbiological parameters, so as to effect direct process control, which is not possible when using microbiological data, as these are only available after a time lag of days.

Each of the many potential sources of risk considered during fault prevention analysis requires individual evaluation.

As an example, the following section describes some of the potential sources of risk concerning preservatives in the manufacture of cosmetics. Product formulation and manufacturing documentation need comprehensive and thorough checking. This includes the documentation of one or more preservative challenge tests, in-use tests etc., manufacturing instructions, including detailed instructions for the processing of preservatives, raw material data sheets with instructions for screening incoming raw materials for stability and storage life and conditions. The receipt of goods, their storage and weighing need to be checked for possible sources of error: whether confusion might arise between different raw materials, or incorrect weighing could result in under- or overdosing of a preservative. The possibility of cross-reactions with other raw materials in the mixture has to be excluded. For products that require a heating phase during manufacture, particular care must be taken to avoid preservatives coming out of solution during cooling. Vice versa, cooling phases during manufacture carry the risk that preservatives may be incompletely dissolved, resulting in possible under-dosing.

Such potential risks should be registered, noted and included in the instructions for manufacture. In this way it can be decided at which point in the production process, and under which physical/chemical conditions (e.g., temperature, pH, concentration of raw material solution) preservatives should be added: for example, intermittently high pH during manufacture can cause the hydrolytic denaturation of Parabens. Depending on dosing, and heating and cooling phases, lipophilic preservatives can migrate into the lipid phase of an emulsion. Some preservatives are heat-sensitive and inactivated by high temperatures. Adding preservatives to too high protein solutions can also cause the inactivation of some preservatives.

The possibility of interactions between primary packaging materials and a preservative, and the latter's inactivation, cannot be ruled out, and should be fully explored and documented during product development.

Hygiene conditions and procedures play a central role throughout the entire manufacturing process. With the finished product, the central issues are checks of microbe-content, analytical checks of preservative content and product release procedures. The case described here makes no claim to be a complete survey of potential sources of risk arising during the manufacture of cosmetics, and has been restricted to preservatives for the sake of brevity. The list could, and should, be expanded to include all other potential areas of risk during manufacture, such as those that might cause a danger to consumer health, or render a product unsaleable.

All the potential risks identified are collated and checked to determine whether direct process control at one of the described process steps would be effective. Should this be the case, then these risks are designated as critical control points.

After documentation of critical control points, procedures are laid down for their surveillance (control conditions), permitted tolerances are set, corrective procedures in the event of deviation from these limits decided upon, and responsibility for the maintenance of procedures assigned. After setting and accepting priorities for the enactment of procedures, the status of the conversion is checked by audit. As long as no further need for additional changes is detected, general and standard operation procedures are committed to record. A further audit is then carried out after approximately one year, or in the event of process or product changes.

Experience with fault prevention studies has shown that this pro-active intervention delivers excellent process optimisation and can pre-empt the occurrence of potential faults. Even if the results of such studies are only ever applied to a single production plant and product, they provide essential knowledge that can be applied to similar production plants.

5.9.8 Summary

In cosmetic products, microbicides are principally used as preservatives, mainly focusing on the protection of water-containing products, the very nature of which provide the basic conditions for the growth of micro-organisms. Securing validation of preservative effectiveness, using the appropriate tests, is vital during the development phase of cosmetic products. This involves *in vitro* preservative challenge tests and in-use testing under conditions of practical use by consumers. To date, virtually no legislation exists for the control of test design and results evaluation. Cosmetics manufacturers themselves bear responsibility for the products they market, ensuring that with use as intended, their products represent no risk to health. Manufacturers are at liberty to decide the manner in which they substantiate the safety of their products. Official instructions merely deal with adherence to the limits for micro-organism content in the finished product and the absence of pathogenic micro-organisms.

Microbiological aspects and their consequences have to be dealt with during the development phase of a formulation, selecting and validating the appropriate preservative system for the product. That cosmetics by their very nature come into contact with the skin or mucous membranes places particularly high demands on the dermatological and toxicological safety of the preservative system. It is obvious that unfavourable skin reactions to cosmetics should be avoided at all cost; this in turn requires that preservative concentrations are as low as practicably possible and, when applicable, preservative 'cocktails' are used. In some cases, the anti-microbial potential of other ingredients can be exploited to ensure sufficient preservative cover.

The use of microbe-sparse raw materials, including production water, combined with good operational hygiene applied to all processes in the manufacture of products, help to eliminate potential sources of contamination and maintain high product quality developed at the formulation stage. In addition, the manufacturer of cosmetic products must also take user-behaviour into account, such that the user can expect enduring product quality right up to the 'use-by-date', even in already opened containers. This is only possible if product preservation in the finished product exercises sufficient preservative capacity. Checking for total viable microbe count, regularly undertaken to verify the microbiological purity of a product, is, however, merely a 'snapshot' of product preservation and provides little information about the true, full-term preservative capacity of a product. Genuinely new products should always be checked by challenge test after manufacture to make sure that product preservation is in no way compromised during any stage of production. As an additional tool in individual cases, fault prevention analyses can be used to detect potential sources of risk during manufacture.

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5.10 Food and beverage preservation

N.N. RACZEK

5.10.1 Introduction

Nowadays in the western hemisphere large tonnages of high quality food worth billions of dollars (Buzby and Roberts, 1997) are ruined due to microbiological spoilage (Agricultural Economic Report, 1997). Spoiled food not only harms the image of food producers but micro-organisms of many kinds are also potentially harmful to the health of consumers. Although the number of people who are really starving is quite low, the subsequent costs for the national economies, especially health services as well as for producers are quite high. On the other hand, in fast developing nations the situation is not comparable: It is frequently not necessary to protect food against deterioration, because fresh food is still available and the food chain is very short; long shelf lives are not required. But developing structures, changing circumstances and the economy will force these societies to use food with longer shelf life as already common in industrialised countries. Additionally in some regions of these countries deteriorated food will intensify the problem of sufficient food-supply. Microbial growth in food leads in developing countries to food shortages with all their ethical impacts and influence on already weak healthiness, due to e.g. mycotoxins. World-wide, the population explosion has resulted in an insufficient supply with good food (Hughes, N., Parsons, N., 2002), which demands a reduction of losses due to microbiological spoilage. Food is not only an agriculture or trade commodity it is a public health issue as well (Käferstein, F., Abdussalam, M., 1998).

World-wide extensive changes in legislation, the attention by governments and increasing consumer interest in microbiological safety and quality of foods became a major subject in everybody's life in the last decade. Numerous micro-organisms, which may contaminate food, may be pathogenic or even toxic, especially for weak people (Mead et al., 2000). Microbiological hazards e.g. with bacterial pathogens associated with foodborne disease are found in weekly warnings all over the world: *Aeromonas hydrophila*, *Bacillus cereus*, *Brucella* spp, *Campylobacter* spp, *Clostridium botulinum* and *perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Plesiomonas shigelloides*, *Salmonella* spp, *Shigella* spp, *Staphylococcus* spp, *Vibrio* spp, *Yersinia enterocolitica* and innumerable mycotoxic fungi are of main interest (Hocking, 1997). Several organisms have been known for centuries to occur on food, some of them are quite new as food spoilage organism (Medeiros, et al., 2001; Bean et al., 1997). Unfortunately most of them are not monitored, the grey numbers of incidences are enormous (WHO, 2000).

Losses and risks can be largely avoided by taking preventive actions, using inactivating processes as well as suitable preservation methods for growth-inhibition. Preventive measures based on quality management and thorough raw material checks in combination with HACCP-concepts developed very positively with honourable effects on microbiological status of intermediate products in the last decades (Notermans and Lelieveld, 2001; Taylor, 1998). Inactivation methods, often based on physical methods such as high pressure treatment, ohmic heating, pulsed light or more traditional ones like cooling, direct heating or drying, may sometimes be used. But these are not suitable for all types of food, and their use may prove to be expensive and doesn't avoid later or cross contamination. The method of choice in this case is to add a preservative. Only the preservatives added can slow or suppress the growth of micro-organisms during the whole process and furthermore control the food when it has already left the direct control of the producer. This gives the producer and the consumer a safety effective in different directions.

Preservatives must be non-irritant, have low acute and chronic toxicity properties and should not decompose into substances more toxic than the preservative itself. They should not retard the activity of digestive micro-organisms and enzymes or affect other intestinal functions causing harm to the consumer. All currently used food preservatives underwent thorough checks by several experts and countless tests in different countries. This is the reason why some 20 years ago the use of a number of preservatives was restricted or even completely forbidden. Therefore today only a handful of preservatives are allowed world-wide, in a broad spectrum of food applications. Some are restricted to special applications or only approved in low concentrations to reduce the possible daily intake. Others are only allowed in some regions, where their use may have also a traditional character supporting other functions. Food producers can still select different preservatives which are active enough to protect well manufactured food, to protect the consumer against deteriorated or spoiled food. The consumer can be sure to obtain unspoiled, good food, containing intensively checked, harmless preservatives.

Extending the shelf life of foods, feeds or other products for daily use of man, retaining the wholesomeness, nutritive and functional value, preventing product decomposition and deterioration and ensuring safety by suppressing the growth of pathogen micro-organisms, is the goal of today's application of preservatives. As a result protection by preservatives will remain very important for everybody's life in the future.

5.10.2 Preservatives

5.10.2.1 Benzoic acid [II, 8.1.9.]*

History. Benzoic acid is one of the oldest chemical preservatives to be used in food. The preservative action of benzoic acid was first described in 1875 by H. Fleck (Strahlmann, 1974; Thorne, 1986). It was not until the turn of the century that it was first introduced for food preservation. Because of its better handling and due to its low price mainly the sodium salt of benzoic acid has become one of the most-used preservatives throughout the world. Nowadays the food industry is striving to reduce the total amount of sodium in the finished food product by using potassium salts as alternatives to the more frequently used sodium form. Although very small growth rates during 1995–2000 because of usage cut-backs (mainly in some beverages) and recent perceptible trends towards restricting its use in favour of other preservatives, considered to be better from the toxicological viewpoint, benzoates still belong to one of the main preservatives used in food applications. Besides its use as a food preservative benzoic acid is used in flavours and perfumes and, in huge amounts, partially in lower quality, for plasticizers and as retarder for rubbers and latex (Maki, 1999).

Names

Benzoic acid

CA Index Name: Benzoic acid.
 Other Names: Acidum benzoicum; Benzenecarboxylic acid; Benzene formic acid; Carboxybenzene; Phenylformic acid; Dracylic acid; Phenyl carboxylic acid; Purox B; E 210.
 Foreign languages: German: Benzoessäure, French: Acide benzoïque, Spanish: Acido benzoico.

Benzoic acid – sodium salt [II, 8.1.9a.]

CA Index Name: Benzoic acid, sodium salt.
 Other Names: Sodium benzoate; Antimol; Benzoate sodium; Benzoate of soda; Sobenate Benzotron; Purox S; E 211.

Benzoic acid – potassium salt [II, 8.1.9b.]

CA Index Name: Benzoic acid, potassium salt.
 Other Names: Potassium benzoate; E 212.

Benzoic acid – calcium salt [II, 8.1.9c.]

CA Index Name: Benzoic acid, calcium salt.
 Other Names: Calcium benzoate; Benzocalol; Calcium dibenzoate; E 213.

Health aspects, acute toxicity. Benzoic acid is absorbed in the human body from the intestine and metabolised through enzymes to hippuric acid (benzoyl glycocholl), which is excreted in the urine. Additionally relatively small quantities of benzoic acid are linked to glucuronic acid and excreted in the urine by this route. Pollution arising from cooking fumes of food preserved with benzoic acid is under discussion (Benfenati, et al., 1998).

LD₅₀ for rats after peroral administration: 1.7–3.7 g/kg body weight (Deuel et al., 1954; Sado 1973). LD₁₀₀ per os for guinea pigs, rabbits, cats and dogs: 1.4–2 g/kg body weight. Cats appear to be especially sensitive to benzoic acid (app. 0.3 g/kg body weight showing a harmful or even fatal effect (Bedford and Clarke, 1972)).

The ADI-level for benzoic acid and its salts is temporarily set (by the Joint expert committees of the WHO/FAO) to 0 – 5 mg/kg body weight/day (WHO, 1997).

Antimicrobial action. The action of benzoic acid is directed mainly against yeasts and moulds. Bacteria are only partially inhibited (Balatsouras and Polymenacos 1963; Uraih et al., 1977).

Benzoic acid has a relatively high dissociation constant (6.46×10^{-5}), which means that benzoic can be used for preserving strongly acid products only. Otherwise a big part is wasted as undissociated, ineffective form (Eklund, 1985).

The microbiostatic action of benzoic acid is based on different inhibition mechanisms, mainly many enzymes in the microbial cell are inhibited (Bosund, 1962; Menon et al., 1990). E.g. in yeast, enzymes that control the acetic acid metabolism and oxidative phosphorylation are inhibited. Benzoic acid appears to intervene at various points in the citric acid cycle, especially that of α -ketoglutaric acid and succinic acid dehydrogenase. Besides its enzyme-inactivating effects, benzoic acid also acts on the cell wall. The types of action of benzoic acid are sometimes very similar to those of sorbic acid, although many more data exist for the latter.

No resistance in the true sense of the term occurs, i.e. there is no rapid increase in the minimum inhibitory concentration under the influence of sub-threshold benzoic acid concentrations. But high tolerance-levels are

* see Part Two – Microbicide Data

described for selected organisms like *Candida*, *Torulopsis* spec. or *Zygosaccharomyces* (Steels et al, 1999). Benzoates can be metabolised by some bacteria employing a pathway involving β -ketoadipate (Chipley, 1993; Sofos 1994).

Applications. In most countries of the world benzoic acid and sodium benzoate have been permitted for food preservation for many years. Apart from a few exceptions, the maximum permissible quantities are between 0.01 and 0.05%. In the USA benzoic acid and sodium benzoate are considered GRAS up to a maximum of 0.1%.

Benzoic acid is used in the form of sodium benzoate mainly to preserve fruit juices intended for further processing. In general, sodium benzoate is sometimes still combined with small quantities of SO_2 in order to protect these products against oxidation and enzymatic spoilage. The concentration of sodium benzoate applied is 0.015–0.05%, depending on the type of juices and the length of time, for which it is desired that the products should be kept fresh (Thouars, 1999). In foods with sensitive flavour such as peach or lemon based fruit drinks benzoates may impart a disagreeable taste at concentrations from 150 ppm. This taste is described as burning and pepper-like aftertaste.

Due to its weak action at neutral pH-levels and its unfavourable distribution coefficient between fat- and water-phases, benzoic acid has lost its importance for preservation of spreads. But it remains important in the preservation of condiments, sauces, mayonnaise and mayonnaise-containing delicatessen products (Flygh and Moeller, 1989; Gonzalez, et al. 1999). In this instance sodium benzoate is usually employed in combination with potassium sorbate, because a better effect against acid producing bacteria can be achieved. Moreover, the potassium sorbate content renders the mixture less obtrusive organoleptically than sodium benzoate on its own.

Benzoic acid is also used for preserving pickled vegetables, an application for which it is well suited owing to the low pH of these products.

5.10.2.2 Sorbic acid [II, 8.1.5.]

History. Comparatively late in 1940 Müller in Germany and Gooding in the United States independently discovered the antimicrobial activity of sorbic acid, although sorbic acid has been known since its isolation out of the oil from the juice of unripe rowan berries by Hofmann in 1859. First industrial supplies of sorbic acid and its salts became available during the late 1950's. Because it is harmless from a physiological standpoint and exhibits favourable sensory characteristics sorbic acid has in the meantime become the leading preservative for a very broad range of food applications. Due to its positive properties a moderate growth of its use in the late 1990's was observed and can be expected for the future as well.

Names

CA Index Name: 2,4-Hexadienoic acid
 Other Names: trans-trans-2,4-hexadienic acid, 2,4-hexadienoic acid, Panosorb, Sorbistat, E 200.
 Foreign languages: German: Sorbinsäure, French: Acide sorbique, Spanish: Acido sórbico.

Sorbic acid – potassium salt

CA Index Name: 2,4-Hexadienoic acid; potassium salt, (2E, 4E).
 Other Names: Potassium (E,E)-sorbate; Sorbic acid, potassium salt; Potassium (E,E)-hexa-2,4-dienoate; Potassium sorbate; Vinosorb; E 202.

Sorbic acid – calcium salt

CA Index Name: 2,4-Hexadienoic acid, calcium salt, (2E, 4E).
 Other Names: Calcium (E,E)-sorbate; Sorbic acid-calcium; Calcium sorbate; E 203.

Sorbic acid – sodium salt. Although the sodium salt of sorbic acid is mentioned in different food regulations it was never used to such an extent as its potassium or calcium salt. Because of its instability it cannot be stored for longer periods. This is the main reason that it is not produced on industrial scale (Lueck et al., 1998).

Health aspects, acute toxicity. Sorbic acid undergoes beta-oxidation in the human body, which is typical for fatty acid degradation (Lang, 1960). Also in higher dosages sorbic acid is mainly expired as CO_2 (rats). No sorbic acid is excreted via urine. The allergenic potential of sorbic acid is considered as extremely low (Haeberle, 1989; Vieths et al. 1994). Sorbic acid and potassium sorbate showed no activity in genotoxic potential in the hamster embryo fibroblast micronucleus assay, and in the cell transformation test in vitro (Schiffmann and Schlatter, 1992), in cultured hamster cells, in the somatic cells of *Drosophila melanogaster* (Schlatter et al., 1992), as well as in other tests (Jung, 1992; Wurgeler, 1992).

The LD_{50} for rats after oral administration is 7.4 – 10.5 g/kg body weight (Deul et al., 1954; Sado, 1973).

An ADI-level for sorbic acid and its salts is fixed (by the Joint expert committees of the WHO/FAO) to 0 – 25 mg/kg body weight/day.

Antimicrobial action. Sorbic acid has a broad activity mainly against yeast and moulds. Its action against bacteria is limited. Catalase-positive bacteria (e.g. Staphylococci, Bacillus spp.) are inhibited to a higher degree than catalase negative (i.e. lactic acid bacteria) ones (York and Vaughn, 1955).

Sorbic acid has a very low dissociation constant (1.73×10^{-5}), which means that it can be used also in food with relatively high pH-levels. The microbiostatic action of sorbic acid can be explained mainly by its inhibitory action on different enzyme mechanisms. In particular enolase, lactatdehydrogenase and several other enzymes from the citric acid cycle and free sulfur-groups containing enzymes are inhibited in the cell (York and Vaughn, 1964; Rehm, 1967). Additionally sorbic acid acts also on the cell membrane (Eklund, 1981 and 1985; Stratford and Anslow, 1998).

No resistance in the true sense of the term occurs, i.e. there is no rapid increase in the minimum inhibitory concentration under the influence of sub-threshold sorbic acid concentrations (Vinas et al., 1990).

Applications. Sorbic acid and its salts are permitted as preservative in all countries of the world in a very broad spectrum of food, pet-food, feed, pharmaceutical and cosmetic products. The permissible quantities are between 0.1 and 0.2%, depending on the application. In the US sorbates can be used in many foods according to GMP without an upper limit. Thus sorbates are some of the most widely used food preservatives in the world.

Depending on the type of food, targeted protection area, process, packaging process and others, sorbates can be added directly in the process or to an intermediate product. They also can be applied through spraying or immersing the product in a water based potassium sorbate solution or an organic solvent of sorbic acid like in alcohols or oils, or by dusting the product with powdered sorbates.

Sorbic acid is used in a variety of bakery products. It has a powerful action against some special moulds occurring on bread (Wallhaeuser and Lueck, 1970). The use of sorbic acid creates no problems, when used in combination with baking powder. The good action of sorbates against yeast can, in the concentration used (0.1–0.25% on dough), adversely affect the leavening of bread. A special form of pure sorbic acid has been developed which dissolves very slowly during dough preparation and does not affect the leavening process (Humbert et al., 1999; Raczek et al., 2003). Sorbic acid is sometimes used in combination with propionates to suppress their bad sensory properties and to gain a broader spectrum and more effectiveness against bread deteriorating micro-organisms. Due to its powerful action against osmophilic yeast, sorbates are also used in various fillings for fine bakery products.

Sorbic acid can be used to preserve ready to eat fruits in concentrations around 0.05% and fruit pulps combined with sulfur dioxide by the addition of about 0.15% potassium sorbate. Sorbates are also used in juice and soft drink production, especially products intended for further processing. In juices 0.05 to 0.2%, in soft drinks around 0.04% of potassium sorbate are applied (Raczek, 1998a). A special quality of potassium sorbate is also used to avoid a secondary fermentation in wine with residual sugar (Raczek, 1998b).

Due to its favourable distribution coefficient sorbic acid is the preferred preservative for fat containing food, like margarine or other fat-emulsions like mayonnaise or dressings. In the latter case potassium sorbate is often combined with sodium benzoate.

Various salts of sorbic acid can be used as surface protection (calcium sorbate, sorbic acid itself) and for the protection of the whole loaf (potassium sorbate) (Raczek and Kreuder, 2000).

Sorbates can be used in cured and uncured meat products (Robach and Sofos, 1982; Saha and Chopade, 2002). Different investigations showed a good inhibition against special micro-organisms resulting in a reduced level of added nitrite in cured meat products. Sorbic acid has also a good preserving action on fresh fish in combination with conventional measures like salting and cooling as well as on dried fish to avoid mould attacks (Thakur and Patel, 1994).

5.10.2.3 Propionic acid [II, 8.1.3.]

History. The usage of propionic acid as an antimicrobial substance in food has been known since the late 1930's and was described by C. Hofmann, G. Dalby and T. R. Schweitzer. Because of its corrosive nature, propionic acid itself is rarely used in the food industry in general, its main field of use having been the feed sector for more than 60 years. Propionic acid is used as an esterifying agent, in production of cellulose propionates. The calcium salt is also used to improve scorch resistance and processability of butyl rubber. Main products for food applications are its sodium and calcium salts, yielding the free acid within the food at low pH ranges. Since its discovery propionates have been mainly used in the preservation of bakery products. Due to some difficulties associated with off-flavors and an increasing number of in-store bakeries the consumption began to stagnate in the food sector in 2001.

Names

CA- Index Name:

Propionic acid.

Other Names:

Ethancarboxylic acid; Propianoic acid; Ethylformic acid; Methylacetic acid; Luprosil; Prozoin; Tenox P; E 280.

Foreign languages:

German: Propionsäure, French: Acide propionique, Spanish: Acido propiónico.

Propionic acid - sodium salt

CA Index Name:

Propionic acid, sodium salt.

Other Names:

Sodium propionate; Sodium ethanecarboxylate; Sodium propanoate; Propanoic acid, sodium salt; Whit-Bioban-S; Deketon; Impedex; Mycoban; Napropion; Ocuseptine; Propi-Ophtal; Propiofar; Propion; PropisolPro; E 281.

Propionic acid - calcium salt

CA Index Name:

Propionic acid, calcium salt.

Other Names:

Calcium dipropionate; Calcium propanoate; Calcium propionate; Propanoic acid, calcium salt; Bioban-C; Molagen; E 282.

Health aspects, acute toxicity. Propionic acid is absorbed by the digestive tract on account of its good water-solubility. Propionic acid is metabolised in the human body in a manner similar to that of fatty acids. Therefore the decomposition products of propionic acid in the tissue of mammals are CO₂ and H₂O. Even after the administration of large doses in the diet, propionic acid is excreted in the urine and there is no risk of accumulation in the human body. High and extensive feed may lead to hyperplasia and severe inflammatory lesions in the forestomach mucosa of rats. Under normal circumstances propionic acid was not observed to have any effect on the mucosa (Harrison, 1991; Bueld and Netter, 1993).

LD₅₀ for rats after peroral administration: 2.6 g/kg body weight, according to other sources: 4.3 g/kg. The acute toxicity of sodium and calcium propionate is in the same range and is not influenced by the combination with other preservatives (Sado, 1973).

Propionic acid irritates the skin and mucous membranes.

Antimicrobial action. The action of propionic acid is directed mainly against moulds. Yeasts are likewise inhibited, and so also are some gram-negative bacteria. Some yeasts (e. g. torula species), are capable of utilizing propionic acid in their metabolism. Inhibition of E. coli may be reversed with addition of β-alanine, indicating that propionates interferes with β-alanine synthesis (Doores, 1993). In general the action of propionic acid is weak in comparison with other preservatives from the organic acid type (Heseltine, 1955).

Propionic acid accumulates in the micro-organism cell and blocks metabolism by inhibiting enzymes. Propionic acid also inhibits growth by competing with other substances necessary for the growth of the micro-organism, especially with alanin and other amino acids. With propionic acid, like other acid based preservatives, the pH value of the material to be preserved is of great importance to the antimicrobial action. Owing to its low dissociation constant, propionic acid behaves in this respect in a favourable manner resembling that of sorbic acid. It can therefore be used for preserving foods with a high pH value (Eklund, 1985).

The fact that propionic acid inhibits bacillus mesentericus, the bacterium that causes rope in bread, is of practical importance in its usage in bakery products.

Applications. The use of propionates for the preservation of bread or certain other bakery products is allowed in virtually all countries with industrialised bread production with limits around 0.2%. In the USA propionic acid, sodium and calcium propionates are considered GRAS and are permitted as an antimicrobial agent or a flavouring agent to GMP in non-standardised foods. A major and very important range of use of propionates is its usage in several feed applications where it is allowed as preservative generally without limited concentration.

Propionic acid is mainly used as its calcium salt in sliced bread and cakes of all kinds and other bakery products susceptible to mould attack (Reiss, 1976). Calcium propionate remains effective in the high pH range of a bread product and is effective against special deteriorating moulds occurring in the bakery sector. Relatively high amounts of propionates are necessary to achieve a sufficient preserving action. Unfortunately those concentrations give the bread a distinct odour, which is most marked in sliced bread.

Furthermore, since the action of propionates against yeast is relatively weak, they do not unduly inhibit leavening in bakery goods raised by yeast, if added in low concentrations (Pollach et al., 2002). Nevertheless, the low absolute efficacy of the propionates means that relatively large concentrations are needed in order to keep bread and other bakery goods free of mould for more than a few days.

5.10.2.4 Sulfur dioxide, sulfites [II, 8.2.2.]

History. Sulfur dioxide was already mentioned in Homer's Odyssey (about 800 B.C.) as a disinfecting smoking agent. The ancient Romans and Egyptians knew the "vapour coming out of sulfur" as an agent improving their wines. Indeed, the fumes from burning sulphur contain gaseous sulphur dioxide which was used in whole ancient Europe where food was stored. The excessive use of sulphur dioxide led in Germany in 1487 to the first restriction as a food ingredient. Therefore the amount of sulfur in wine barrels was restricted and additionally only one single dosage was allowed (Strahlmann, 1974). Due to several other effects (e.g. antioxidant, enzyme inhibition) this probably oldest preserving agent for food is nowadays still in use. Worldwide there are ongoing efforts to reduce the amount of sulphur dioxide in food due to the questionable toxicological behaviour and organoleptic changes associated with its use. The usage of sulphurdioxide and sulfites for other applications as for paper pulp, metal refining, bleaching, sulfonation, etc. are economically more important than the application as food preservative.

Names

CA Index Name: Sulfur dioxide.
 Other Names: Sulfurous anhydride; Sulfurous oxide; E 220 (sodium metabisulphite: E 223, sodium sulphite: E 221)
 Foreign languages: German: Schwefeldioxid, French: Dioxyde de soufre, Spanish: Dióxido de azufre.

Health aspects, acute toxicity. Sulfite oxidase in human liver and kidneys leads to an oxidation from sulfite to sulfate. Sulfate is rapidly excreted in the urine. Therefore there is no accumulation in the human body. Fatal poisoning per os with sulfur dioxide is nearly impossible, because vomiting occurs.

LD₅₀ for rats after peroral administration: 1.0 – 2.0 g/kg body weight. For rabbits LD₅₀ per os was determined between 600 and 700 mg/kg body weight for cats as 450 mg (Jaulmes, 1970).

Several different intolerance reactions like urticaria, asthmatic attacks and induction of allergies and pseudo-allergic reaction in humans are known (Tsevat, 1987; Acosta, 1989; Simon, 1993). Sulfites irritate the skin and mucous membranes (Wuthrich, 1993).

An ADI-level for sulfites, calculated as sulfur dioxide, is fixed (by the Joint expert committees of the WHO/FAO) to a maximum of 0 – 0.7 mg/kg body weight/day.

Antimicrobial action. Sulfur dioxide and the sodium and potassium salt of sulfite, bisulfite and metabisulfite all appear to act similarly and are treated here together. The action of sulfur dioxide is mainly directed against bacteria, lactobacilli being especially sensitive to sulfur dioxide (Carr et al., 1976). The action of sulfur dioxide against yeast and moulds is limited. Different types of yeast and moulds are inhibited by very different concentrations. The main cause of its action is the reaction with amino acids containing SH-groups. Therefore a number of enzymes are completely inhibited, and alterations in proteins, nucleic acids and in prosthetic groups take place. These reactions have a massive influence on different metabolic systems like energy production, protein biosynthesis, DNA replication, membranes and others. Due to its dependence upon the surrounding pH-level, which can be described in the form of undissociated sulfurous acid (low pH-level), hydrogen sulfite and sulfite ions (high pH-levels), the antimicrobial action can vary in a broad concentration-range (King et al., 1981). The dissociated sulfite ions have no antimicrobial effect, whereas the sulfurous acid or dissolved sulfur dioxide gas have the most powerful effects. But in the main application area, wine, the main proportion is bisulphite ions, with only 5% in active molecular form (Romano and Suzzi, 1993).

Applications. Sulfur dioxide is used in its gaseous or liquid form, or in the form of one of its salts mainly on dried fruits, molasses, syrups, concentrates, fruit juices and wines. Sulfur dioxide, sulfites, bisulfites and pyrosulfites are more or less equivalent permitted as active ingredients in food worldwide. Many countries do not allow the use of sulfite for meat, fish or processed meat and fish products anymore.

Of great importance is its application in wine against a broad range of spoilage organisms (Toit and Pretorius, 2000). Therefore sulfur dioxide is added to the grape-must (40–50 mg SO₂/L) where acetic acid bacteria and undesired yeast are inhibited (Rose, 1993). Later on in the process also acid degrading bacteria are inhibited. The use of sulfur dioxide to stop the fermentation process in wine is an undesired application because of the high concentrations (up to 2 g SO₂/L) in the endproduct (Delfini and Formica, 2001). Another positive effect is the binding of acetaldehyde which would give the wine an undesired flavour.

In fruits or vegetables for further processing (e.g. for jam) sulfur dioxide is reduced due to a heat treatment, or a vacuum during processing, so that its concentration in the endproduct is limited. In raisins 1 g SO₂/kg is added and in other dried fruits up to 2 g SO₂/kg can be found. In these applications sulfur dioxide has an antioxidant

function to prevent browning. For this reason it is also used in fresh fruit and various vegetables because sulfites inhibit most oxidising enzymes like peroxidases and lipoxygenases. In some fermentation processes the addition of sulfur dioxide has no influence on the fermentation flora and quality of the endproducts (Jang Gong Liu et al., 2001). Food containing thiamin is normally not preserved with sulfur dioxide because it decomposes this vitamin.

Sulfites are active against bacteria which can be found on meat (Kidney, 1974). Also in this application field a colour stabilising effect occurs which, however may lead to a false impression of freshness.

5.10.2.5 Nitrite, nitrate

History. Nitrites and nitrates, mainly the sodium and the potassium salt, have been used in food preservation already since the start of the Christian era in cheese, fish and meat (Binkerd and Kolari, 1975). It was first realised in 1899 that the active agent is the nitrite-ion which is formed microbiologically. The conversion of nitrate to nitrite is a natural and therefore an uncontrolled process. Since that moment there has been an increasing inclination for the direct use of nitrites. Nowadays nitrite is solely used in mixtures, mainly with common salt. This is done to make dosage simpler and more reliable. Nitrates are mainly produced for other than food industries applications like explosives, matches, fertiliser, glass manufacturing. Nitrites are also used as colour fixative. In view of the toxicological properties of the nitrite itself and of the nitrosamines formed in cured food, the continued use of nitrites in food preservation was undergoing a critical investigation for the last decades (Walker, 1990; Derache and Derache, 1999; Hernandez et al., 2001). Therefore, the use of nitrates is also declining, but in the nearest future they will remain important concerning its colour stabilising effects especially for meat products, because substitution products are still in the phase of development.

Names

Sodium nitrite

CA Index Name:

Sodium nitrite.

Other Names:

Nitrous acid sodium salt; E 250; mixture with common salt: nitrite pickling salt, curing salt.

Foreign languages:

German: Nitrit, French: Nitrit, Spanish: Nitrito.

As mixture with common salt: German: Nitrit-Pökelsalz, French: sel nitrique de saumure, Spanish: sal de salmuera nítrica.

Potassium nitrate

CA Index Name:

Potassium nitrate.

Other Names:

Niter; Nitre; Saltpeter; E 252.

Foreign languages:

German: Salpeter, French: Salpêtre, Spanish: Nitratos.

Health aspects, acute toxicity. Nitrates cause local intestinal irritations and diarrhoea owing to dehydration in the intestine. The lethal dosis for humans is around 30 mg/kg. Nitrates uptaken with food are converted into nitrite in the intestine. Nitrites are readily absorbed by the intestinal tract. In infants this conversion already takes place in the stomach or duodenum where nitrite is absorbed and can lead in higher dosages to cyanosis. The toxicity of nitrite has led to poisoning when nitrite has been used improperly as such or mistakenly used instead of nitrate in meat. With amines nitrites can build nitrosamines under acidic conditions, nitrates build N-Nitroso compounds (after long term consumption) and both have carcinogenic properties (Derache and Derache, 1999; Levallois et al., 2001; Archer, 2001). Irradiation (> 10 kGy) can decrease nitrosamines and nitrite concentration in sausages (Ahn et al., 2002).

The LD₅₀ of sodium nitrate is between 3 and 7 g/kg bodyweight, sodium nitrates LD₅₀ lies between 0.1 and 0.2 g/kg bodyweight (rats).

The ADI-level for sodium-and potassium nitrate is fixed to a maximum of 0 – 5 mg/kg body weight/day, the ADI-level for sodium-and potassium nitrite is fixed to a maximum of 0 – 0.1 mg/kg body weight/day (except for baby-food) by the Joint expert committees of the WHO/FAO.

Antimicrobial action. The action of nitrates and nitrites is very limited. The growth of moulds and yeast is not affected by nitrites. Main goal is the prevention of growth of pathogenic bacteria, especially clostridia and thus a formation of botulinum toxin in meat (Roberts and Smart, 1974; Ruiter, 2001). In dairy products undesired fermentation caused by micro-organisms like clostridia, coli or butyric acid bacteria can also be inhibited. The antimicrobial action of nitrate results much more from the nitrite produced directly in the food. The antimicrobial action of nitrites is based on the nitrous acid they release and the oxides of nitrogen produced from the nitrous acid. This is the reason why the action of nitrites increases with a falling pH level. Nitrous acid produced attaches to the amino groups of different peptides, proteins as well as to the dehydrogenase system of the micro-organism cell and thus cause an inhibitory action. Another point of attack for the nitrite is the

hemoprotein, such as cytochromes and sulphur containing enzymes (Roberts et al., 1990). The antibacterial effect of nitrites is enhanced in the presence of reducing agents (like cysteine and ascorbate) and with sorbates.

Nitrates are less effective because they must be produced in the food, and a number of micro-organism strains can even use nitrates as a source of nitrogen.

Applications. Nitrites are permitted in a number of countries as preservatives for meat and fish products. Initially nitrite was used alone for this application. But in most countries it is now employed only in a mixture with common salt (and sometimes) sucrose in fixed and often legally stipulated ratios. Main application for nitrate and nitrite are the meat sector. Nitrate is used dry or in solution. In dry curing, liberal quantities of dry curing salt are rubbed into the meat, after which the cuts of meat so treated are placed in pickling vessels and weighted down. In this way the common salt, by means of osmosis, extracts some of the tissue fluid partly covering the meat. Frequently this process is followed by pickle-curing. Nitrites can also be applied under vacuum or ultrasound conditions or as artery or spray pumping. Nitrite concentrations in meat amounting between 0.05 and 0.2 g/kg are not sufficient to gain a reliable protection. But in combination with other hurdles food can be sufficiently preserved (Baird-Parker and Baillie, 1973; Lechowich et al., 1978). The desired effects, besides its antimicrobiological activity, are the stabilisation or changes in the meat's colour, antioxidative effects and a positive influence on the flavour. These effects can be judged as positive side effect, which as in the case of SO₂, has a big impact on its use, although there are some toxicological doubts. Substitution products like sorbic acid and its salts have in some cases even a superior antimicrobial action to that of nitrites (Robach and Sofos, 1982; Lueck, 1984). But they never became established in this area due to the missing colour and flavour influence. Nitrate is converted into nitrite which is virtually not detectable in the end product. The drawback of employing nitrate is that its conversion into nitrite is uncontrolled. Some countries still allow the use of nitrates in vat milk to prevent blowing in cheese production. Higher concentrations can lead to discolouring in this application.

5.10.2.6 Esters of *p*-hydroxybenzoic acid (parabens) [II, 8.1.11.]

History. In the beginning 1920's T. Sabalitschka synthesised different alkyd and aryl esters of *p*-hydroxybenzoic acid with a view to discover a replacement for salicylic- and benzoic acids, which suffer from the drawback of being effective only in the highly acid pH-range. This is why esters of *p*-hydroxybenzoic acid are still mainly used in non food applications with pH-levels around the neutral, as pharmaceuticals or cosmetics. Due to their negative sensory properties, their toxicological behaviour and some other technical problems associated with their use in foods, the consumption of parabens acid in food remains very small. Additionally esters of *p*-hydroxybenzoic acid are the most expensive of the available preservatives. Therefore an additional growth of the amount used currently in the food sector cannot be expected in the near future.

Names

4-hydroxybenzoic acid methyl ester

[II, 8.1.11.1.]

CA Index Name:

Methyl 4-hydroxybenzoate.

Other Names:

Methyl ester of 4-hydroxybenzoic acid; Methyl parasept; Methyl para-hydroxybenzoate; Methylparaben; Nipagin M; E 218 (sodium salt E 219).

4-hydroxybenzoic acid ethyl ester

[II, 8.1.11.2.]

CA Index Name:

Ethyl-4-hydroxybenzoate.

Other Names:

4-Hydroxybenzoic acid ethyl ester; Carbethoxyphenol; Ethyl parahydroxybenzoate; Ethylparaben; Nipagin A; E 214 (sodium salt E 215).

4-hydroxybenzoic acid propyl ester

[II, 8.1.11.3.]

CA Index Name:

Propyl 4-hydroxybenzoate.

Other Names:

Propyl parasept; 4-hydroxybenzoic acid propyl ester; Propyl parahydroxybenzoate; Propylparaben; Nipazol M; E 216 (sodium salt E 217).

Foreign languages (Esters):

German: *p*-Hydroxybenzoesäureester, French: 4-hydroxybenzoates, Spanish: 4-hidroxibenzoatos.

Health aspects, acute toxicity. All *p*-hydroxybenzoic acid esters are completely absorbed from the gastrointestinal tract and hydrolysed. The *p*-hydroxybenzoic acid formed in the hydrolysis is excreted via the urine.

Some parts are metabolised through enzymes to p-hydroxyhippuric acid, which is excreted in the urine as well. Additionally relatively small quantities of parabens are linked to esters of glucuronic acid and excreted in the urine by this route. Only small concentrations of p-hydroxybenzoic acid are detectable in the blood.

LD₅₀ of the methyl- and propyl-ester in mice feed is around 8 g/kg body weight (for the sodium salts 2 g and 3.7 g/kg body weight), for rabbits some 3 g/kg body weight.

The LD₅₀ (oral) of the ethyl ester is about 6 g/kg mouse (the sodium salt of the ethyl ester has a LD₅₀ oral of 2 g/kg body weight) (Matthews et al., 1956; Sado, 1973).

The ADI-level for every p-hydroxybenzoic acid ester is temporarily fixed to a maximum of 0 – 10 mg/kg body weight/day by the Joint expert committees of the WHO/FAO.

Antimicrobial action. All p-hydroxybenzoic acid esters have principally a fungistatic action. The p-hydroxybenzoic acid esters destroy the cell membrane and lead to a protein denaturation in the interior of the cell; besides competitive reactions occur with coenzymes (Tatsuguchi, et al. 1991). Under aerobic conditions Paraben is hydrolysed to p-hydroxybenzoic acid. The following decarboxylation of which leads to phenol, [II, 7.1.] (Valkova, et al., 2001).

As p-hydroxybenzoic acid esters present a relatively high pK_a value (approx. 8.5), their antimicrobial action is less dependent on the pH value of the type of food to be preserved. In this respect they are superior to the organic preservative acids. Unfavourably, as in the case of phenol, p-hydroxybenzoic acid esters may be linked to some extent to proteins, emulsifiers or other food components on account of their phenolic hydroxylic-group and thus be inactivated. The antimicrobial action of the p-hydroxybenzoic acid esters is proportional to the alkyl chain length. Thus the antimicrobial action of the methyl ester is some 3 to 4 times, that of the ethyl ester some 5 to 8 times, that of the propyl ester about 25 times as powerful as phenol (Thompson, 1994). However, superimposed on this effect may be the water solubility of the esters, which is inversely proportional to the alkyl chain length. Since the activity spectra of the individual p-hydroxybenzoic acid esters are different, the application of mixtures can be profitable.

Applications. In many countries the methyl, ethyl and n-propyl esters of p-hydroxybenzoic acid, as well as their sodium salts, are permitted for preservation of some foods. The maximum permissible quantity is around 0.1%. In the USA methyl- and propyl-paraben are considered GRAS. The maximum permissible quantity is 0.1%. Their main field of use is not food preservation but the preservation of pharmaceutical and cosmetic fat emulsions. The usage of the butyl ester is not allowed in food applications.

The p-hydroxybenzoic acid esters, especially those with long alkyl chain rest, can be used when there is a need to provide a strong protection against micro-organisms in perishable food, but owing to their organoleptic properties and unfavourable distribution between fat and water there are only some special application fields remaining. These are for example its usage in fillings for confectionery, some snacks or bakery goods and in preservative mixtures for fish marinades. In the latter case p-hydroxybenzoic acid esters are often used in combination with benzoic acid and sorbic acid. The applied concentrations rarely exceed 0.05%, relative to the end product, because the esters of p-hydroxybenzoic acid impart a perceptible taste in higher concentrations. Sometimes the esters of p-hydroxybenzoic acid can still be found in meat or other fish products. Especially p-hydroxybenzoic acid ethyl- and propyl-esters are used in concentrations of 0.05 to 0.1% for preserving sausage and meat coatings containing gelatine. Current investigations evaluate the usage of parabens in shellac formulations for surface protection of citrus fruits post harvest (Mc Guire and Hagenmaier, 2001).

The oil solubility varies depending on the nature of the oil, but is the poorest for the methyl ester. In comparison to preservative acids the methyl ester has already a relatively unfavourable distribution coefficient between the oil and water phases in emulsions.

5.10.2.7 Nisin [II, 20.11.1.]

History. At the end of the 1920's L.A. Rogers detected that several lactic acid bacteria are inhibited by metabolites of streptococcus strains. After rediscovery by A.T.R. Mattick and A. Hirsch of this phenomenon in 1944, industrial production of nisin began in the 1950's. Nisin has been used in food preservation since that time on a limited scale for dairy products. During the 1990's a lot of research provided the food industry and health organisations with supporting information, and broader application and usage in industry followed. Nowadays, due to several producers around the globe, nisin is no longer a speciality and moves toward a commodity business, although it is used in the form of exclusively produced, standardised preparations.

Names

CA Index Name:

Nisin A.

Other Names:

1-Thia-4,7,10,13,16,19-hexaazacyclodocosane; 1-Thia-4,7,10,13-tetraazacyclohexadecane; 1H,9H-Pyrrolo[2,1-i][1,4,7,10]thiatriazacyclotridecine; 9,19-Dithia-2,5,13,16,22-pentaazabicyclo[9.9.2]docosane; L-isoleucyl-(Z)-2,3-didehydro-2-aminobutanoyl-D-cysteinyl-L-isoleucyl-2,3-didehydroalanyl-L-leucyl-L-cysteinyl-threo-3-mercapto-D-2-aminobutanoyl-L-prolylglycyl-L-cysteinyl-L-lysyl-threo-3-mercapto-D-2-aminobutanoylglycyl-L-alanyl-L-leucyl-L-methionylglycyl-L-cysteinyl-L-asparaginyl-L-methionyl-L-lysyl-threo-3-mercapto-D-2-aminobutanoyl-L-alanyl-threo-3-mercapto-D-2-aminobutanoyl-L-cysteinyl-L-histidyl-L-cysteinyl-L-seryl-L-isoleucyl-L-histidyl-L-valyl-2,3-didehydroalanyl-, cyclic (3-7),(8-11),(13-19),(23-26),(25-28)-pentakis(sulfide); MicroGARD; Nisaplin; Nisapol; Nisin 1-34; Novasin; Ambicin N; E 234.

Foreign languages:

German: Nisin, French: Nisine, Spanish: Nisina.

Health aspects, acute toxicity. As nisin is particularly sensitive to proteolytic enzymes such as trypsin, pepsin, pancreatin, salivary enzymes and digestive enzymes (except rennet), it is metabolised in the human body into amino acids. Hence, nisin is very unlikely to be toxic to man. Additionally nisin has been present since time immemorial in milk and cheese, and likewise in streptococci, which produce nisin, are regularly present in the intestine (Gudmundsdottir, 1991; Kalra et al., 1992; Delves-Broughton, 1998).

The LD₅₀ after oral administration (mice) corresponds to 6.9 g/kg bodyweight. Dosages of up to 1×10^6 reading units/kg body weight have proved harmless to rats. Doses of 400 mg nisin/kg body weight daily under conditions of stress led to increased mortality in an 8-week feeding experiment with mice.

The ADI-level for nisin is fixed to a maximum to 0 – 0.13 mg/kg body weight/day by the Joint expert committees of the WHO/FAO.

Antimicrobial action. Nisin is isolated from cultures of lactic acid bacteria, e.g. streptococcus lactis or streptococcus cremoris. Consequently it acts exclusively against some gram-positive bacteria, like streptococci, bacilli, clostridia and some other anaerobic spore-forming microorganisms. Yeast and moulds are not inhibited by nisin. On the contrary, many of these micro-organisms tend to decompose nisin. Through interaction with bound cell wall precursors immediately after germination of the spores, the action of nisin is directed against the bacteria membrane. As a result highly specific pores are formed (Breukink, et al., 1999; Wiedemann, et al., 2001). Nisin kills the bacterial target rather than simply inhibiting growth like benzoates or sorbates. Nisin resistant strains did not develop intrinsic high tolerances against low pH-levels, high salt concentrations or common preservatives like potassium sorbate or nitrites (Mazzotta, 2000). Nisin intensifies the heat sensitivity of bacteria spores. Thus, nisin does not attack the spores directly. Its action occurs not during a heating process but afterwards. Combination with other hurdles or substances may lead to an optimised antimicrobial action of nisin (Periago and Moezelaar, 2001; Cerrutti et al., 2001; Lee et al., 2001), but can be influenced by different enzymes (Guerra and Pastrana, 2002).

Applications. Nisin is permitted in some countries chiefly for the preservation of processed cheese and it is used in (fruit- and vegetable-) preserves as a sterilising auxiliary, but not in the United States or the EC. In the EC nisin can be applied in clotted cream, mascarpone, processed and ripened cheese in concentration around 10 mg/kg.

Because of its pH-dependent antimicrobial action (the pH-optimum lies between 6.5 and 6.8, although its stability in this pH range is already poor) nisin can only be used in specific nearly pH-neutral foods without bigger losses of activity. The main varieties to be suppressed e.g. in (processed) cheese, are clostridia and butyric acid bacteria. In this application nisin is added during the melting process, as nisin-producing culture or directly in powder form. Nisin is also active against the so called “late blowing” of hard cheese which is caused by different clostridia spec.. Other fields of application are its usage in liquid egg and preparations thereof, yoghurt and several types of cheese spreads (Tortorello et al., 1991; Rodriguez-Gomez, 1996) and cooked pork (Aymerich, et al., 2002). Usage levels range from 0.2 to 15 mg/kg food. Nisin packaging films for seafood where only effective, if stored at lower temperatures (Young, et al., 2002). Although nisin has a slight action against malaria it is not used in medical applications for humans.

5.10.2.8 Pimaricin [II, 20.11.2.]

History. Pimaricin was first separated out of filtrates of streptomyces natalensis by A.P. Struyk and J.M. Waisvisz in the early 1950's. The polyene antibiotic gained its name from “Pietermaritzburg” in South

Africa, where the sample in which the relevant strain had been discovered was taken. Pimaricin has been used as a vaginal suppository for the therapy of candida infections and for other medical purposes like ophthalmic suspensions (Raab, 1972). Since the early 1960's efforts have also been made to introduce it as a food preservative. Also in this case it has achieved a certain importance especially in the surface preservation of cheeses. Due to the currently increasing world-wide concern about the resistance of pathogenic bacteria against antibiotics it is discussed whether antibiotics used in food should be banned or its usage should be more restricted in the future. The addition of preparations containing pimaricin to cheese coatings based on polymer emulsions is still an important application in this industrial sector.

Names

CA Index Name: 6,11,28-Trioxatricyclo[22.3.1.05,7]octacosane-8,14,16,18,20-pentaene-25-carboxylic acid, 22-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-, (1R,3S,5R,7R,8E,12R,14E, 16E,18E,20E,22R,24S,25R,26S).

Other Names: 6,11,28-Trioxatricyclo[22.3.1.05,7]octacosane; [1R-(1R*,3S*,5R*,7R*,8E, 12R*, 14E, 16E, 18E, 20E, 22R*, 24S*, 25R*, 26S*)]-22-[(3-Amino-3,6-dideoxy-δ-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.05,7]octacosane-8,14,16,18,20-pentaene-25-carboxylic acid; Tenecetin; (δ-) Natamycin; Natamycin; Myprozine; Pimafucin; Natamax; E 235.

Foreign languages: German: Natamycin, French: Natamycine, Spanish: Natamycina.

Health aspects, acute toxicity. Pimaricin is virtually fat- and water-insoluble, though it is not absorbed from the intestines of humans and the majority of ingested pimaricin will be excreted in the faeces. Pimaricin is well tolerated by the skin and mucous membranes and is therefore an ideal substance for topical treatment.

The LD₅₀ of pimaricin after peroral administration has been determined as 1.5 g/kg body weight for the mouse and rat. According to other authors LD₅₀ of pimaricin lies around 0.45 g/kg (guinea pigs) (Levinskas, 1966).

The ADI-level for Pimaricin is set (by the Joint expert committees of the WHO/FAO) to 0–0.3 mg/kg body weight/day.

Antimicrobial action. Pimaricin binds to membrane sterols like other polyene antibiotics inducing distortion of membrane permeability (Oostendorp, 1981). This is why Pimaricin acts against some yeast and moulds and is ineffective against bacteria. The vital activity of cells can be inhibited by substantially smaller concentrations of pimaricin than those required to inhibit the growth of the cells. In particular, pimaricin acts against moulds that grow on human skin; hence its use in medicine. Data on skin fungi have revealed that these gradually become resistant to pimaricin, although no such resistance has been observed among some other varieties of fungi (Athar and Winner, 1971; Dekker and Gielink, 1979). Pimaricin, in combination with high salt concentrations, low pH-levels or temperatures can slow down mycelial growth and toxin production (Rusul and Marth, 1988). Pimaricin is not stable in cheese coatings, depending on the temperature (Engel et al., 1983).

Fields of use. The main application of pimaricin in the food sector is found in the sector of surface treatment of hard cheese and as an additive to cheese coatings based on polymer products, provided pimaricin didn't migrate into the cheese to a specified extend. In general a greater depth of penetration has been observed in soft cheese than in hard. Best preserving results are achieved by using a plastic dispersion with pimaricin alone or in combination with sorbates (Fente, 1995) or propionates (Tortorello, 1991). Pimaricin can also be used for the surface treatment of sausages due to its good action against moulds. In industrialised countries this is the only allowed usage and pimaricin addition is prohibited for use in other foods.

5.10.2.9 Antibiotics [II, 20.11.]

Names

Foreign languages: German: Antibiotika, French: Antibiotiques, Spanish: Antibióticos.

Antibiotics are metabolites produced by micro-organisms that kill or inhibit other micro-organisms. Only two antibiotics have retained some importance, albeit relatively minor: nisin and pimaricin (for details see description above). Three others namely different tetracyclines, subtilin and tylosin have been studied and found effective for various food applications. But these substances have never been widely allowed as a food additive, because substances employed therapeutically shall not be used as food additives. In general antibiotics are prohibited

for food preservation in principle despite being fundamentally suitable from the technical viewpoint, since it is feared that their regular ingestion in food would lead to acquired resistance and hence undesirable influences on their therapeutic application and sometimes lifesaving action. Nevertheless antibiotics are still used as therapeutic agents and feed additives, especially at the beginning of the food chain. But these applications will be more and more restricted or completely banned due to mentioned health risk for humans.

Nevertheless great effort was made in the field of science in the last decade to find antimicrobially acting metabolites from bacteria with good action, mainly against pathogens. Often these substances are isolated out of lactic acid bacteria and belong to the class of Lantibiotics, which also includes nisin (Montville and Kaiser, 1993). As long as these substances are only used in food, the risk of resistance because of its medical application can be banned (Ross et al., 1990).

It is known that the application of different tetracyclines is effective against bacterial spoilage on fish, seafood, poultry, red meat, vegetables, between 7 and 10 ppm on the surface, of the food. Oxytetracyclin and chlorotetracyclin were approved by FDA in the mid 1950's in poultry at 7 ppm levels. But its usage was forbidden again after a short time. Because of their stability, which cannot be destroyed by the usual food processing techniques, undesired effects on the human organism may appear. Misgivings about the toxicological aspects of tetracyclins are essentially of a relatively minor nature. The LD₅₀ of chlorotetracyclin is only some 1.5 g/kg body weight. Hence, the only danger in using tetracyclins for food preservation, i. e. in its continuous ingestion, is the possibility of its affecting the intestinal flora and the mentioned problem of resistance. They are used to treat diseases in humans and animals and are used also in feed supplements. The risk associated with their use as food preservative seem clearly to outweigh the benefits.

Subtilin was discovered in the late 40's by USDA and is closely related to nisin in its structure and behaviour. Like nisin it is only active against gram-positive bacteria. Subtilin has been used by way of experiment in concentrations of 20 ppm to reduce the stringency of the sterilisation conditions for canned foods. It has the advantage that virtually none of it is absorbed; so its influence on the intestinal flora is only slight. Subtilin is, moreover, not used for medical purposes.

Tylosin is a macrolide antibiotic which is likewise not used therapeutically. It has only a very slight acute toxicity, with an LD₅₀ of 12 g/kg body weight. It has a higher activity in comparison to nisin and subtilin, but its effect is also exclusively directed against bacteria. Tylosin has occasionally been used in eastern Asia for preserving fish products and also, like nisin, for improving heat sterilisation. It is still used in some countries to treat animal feed.

5.10.2.10 Dimethyldicarbonate (DMDC) [II, 9.7.]

History. A possible antimicrobial action of different pyrocarbonic acid esters as cold pasteurisation agents of beverages was first described in the late 50's. Since diethyldicarbonate, the precursor of DMDC, which was introduced in the 60's, may be a teratogenic agent and ethyl-urethan, which can be formed out of diethyldicarbonate in a pH-dependent reaction, is a carcinogen, in the beginning 80's diethyldicarbonate was replaced by DMDC in industrialised countries. DMDC forms methyl-urethan which is not carcinogenic. Recent developments in the soft drinks industries have resulted in new types of drinks with a permanently growing number of different ingredients. So the susceptibility of the beverage and the requirements in the production of these types of beverages and the use of cold sterilising agents will grow. A similar growth can be expected for the combination of DMDC with persistent preservatives which remain active in the beverage.

Names

CA Index Name:	Dimethylcarbonic acid ester.
Other Names:	Dimethylpyrocarbonat; Dimethylpyrocarbonic acid ester; Dicarbonic acid dimethyl ester; Velcorin; E 242.
Foreign languages:	German: Dimethyldikohlensäureester, French: Dicarbone de diméthyle, Spanish: Dimetildicarbonato.

Health aspects, acute toxicity. The LD₅₀ of DMDC is 350 – 500 mg/kg body weight (rats) after peroral administration. DMDC causes irritations of the mucous membranes and the skin and must be handled with care.

There is no ADI-level for DMDC because the substance toxicity is of minor importance, since in its application field, beverages, DMDC hydrolyses fast; only its reaction products (mainly methanol) remain. DMDC itself should not be detectable in the finished product. Methyl-carbamate as a minor reaction product is not carcinogenic, mutagenic or teratogenic and is permitted in drinks up to 0.02 mg/l.

Antimicrobial action. DMDC acts as a cold sterilising agent. In comparison to conventional preservatives it acts very fast in combating micro-organisms, but does not provide a long term preservation or protect against

recontamination (Bizri and Wahem, 1994). The antimicrobial action is based on the breakdown of protein and peptide nitrogen in the membrane and enzymes in the micro-organisms. DMDC acts mainly against spoilage yeast. Sometimes some selected bacteria are inhibited (Fisher and Golden, 1998). Its action against moulds is very weak, some yeast show high tolerance (Steels et al., 1999). After DMDC has decomposed there is no longer any microbiocidal effect.

Field of use. DMDC is used in different beverage applications like soft drinks, free of or containing fruit juice, sports drinks, wine or beer as a cold sterilising agent. DMDC is approved in soft drinks, wine and several beverage concentrates in all industrialised countries. The maximum addition level lies between 200 and 300 ml/l. Due to its properties and special dosage technique with a metering equipment the application and operation of the equipment makes trained personnel necessary. To achieve its optimum effect, DMDC must be dispersed uniformly in the beverage. As this is not guaranteed in beverages containing particles, like parts of a fruit, a reliable cold sterilisation of such products with DMDC is not possible. Non carbonated drinks with high pH-levels require high additions of DMDC. DMDC is used in combination with potassium sorbate to take advantage of its synergistic action against some micro-organisms in order to guarantee a long term protection (Anon., 1997).

5.10.2.11 Dehydroacetic acid [II, 8.1.8.]

History. Dehydroacetic acid belongs to the group of younger preservatives. It was discovered in 1947 by G.H. Coleman and P.A. Wolf. Due to its relatively high toxicity it has never acquired great significance. Nowadays it is only used in some Asian countries in very specific applications and its use will not grow. Nevertheless it is a remarkable micobicide on account of its good effect in the high pH range in foodstuffs.

Names

CA Index Name: Dehydroacetic acid.

Other Names: Dehydracetic acid; 2-Acetyl-5hydroxy-3-oxo-4-hexenoic acid delta-lactone; 3-Acetyl-4-hydroxy-6-methyl-pyrone

Foreign languages: German: Dehydracetsäure, French: Acide dehydracétique, Spanish: Acido deshidroacético.

Health aspects, acute toxicity. Dehydroacetic acid is rapidly and completely absorbed by the human body. The acid is dispersed in the plasma and numerous organs. It is excreted in the urine (Parke, 1992).

LD₅₀ for rats after administration per os in the feed in the form of an oily suspension is 1 g/kg body weight.

Corresponding values for the sodium salt of dehydroacetic acid are 570 mg/kg for rats and 400 mg/kg for dogs.

Due to its very small permission in only some countries, there is no ADI-level for Dehydroacetic acid set by international Organisations.

Antimicrobial action. Dehydroacetic acid mainly acts against yeast and moulds. To act against bacteria very high concentrations are necessary, especially against pseudomonas and staphylococcus spec. Against escherichia action of dehydroacetic acid is similar to benzoic or sorbic acid (Yamamura, 2000). Dehydroacetic acid has a relatively low dissociation constant and therefore remains effective up to pH 6. Dehydroacetic acid additionally inhibits various oxidation enzymes.

Field of use. In Europe dehydroacetic acid is not permitted as a food preservative. Dehydroacetic acid was permitted in the USA for treating cut or peeled squash with a maximum permissible residual quantity of 65 mg/kg. In some asian countries dehydroacetic acid is still allowed but its usage is of minor importance (Ishiwata, 1997).

Dehydroacetic acid is used for foods with a relatively high pH value like cheese, margarine, bakery goods, occasionally also in salad dressing and ketchup. Additionally it can be used in combination e.g. with nisin to control listeria on catfish (Degnan, 1995). Because of its toxicity, however, dehydroacetic acid has been unable to achieve world wide importance in any of these fields of use.

5.10.2.12 Thiabendazole [II, 15.9.]

History. Since 1962 thiabendazole has been used in the medical sector for animals as an anthelmintic. Since then it was used in different areas as a fungistatic agent for use in medicine (since 1964) or in crop protection as a postharvest fungicide. It was from this latter application that thiabendazole came to be adopted for food preservation, where it is still used on a limited scale for preserving citrus fruits and bananas surfaces.

Names

CA Index Name: 1H-Benzimidazole, 2-(4-thiazolyl).
 Other Names: Benzimidazole, 2-(4-thiazolyl); 2-(4'-Thiazolyl)benzimidazole; 2-(Thiazol-4-yl)benzimidazole; 2-(4-Thiazolyl)-1H-benzimidazole; 2-(4-Thiazolyl)benzimidazole; 5-(4-Thiazolyl)benzimidazole; Amolden HS; Chemvicon TK 100; Cropasal; Drawipas; Equizole; G 491; Hokustar HP; Mertect; Mertect; Metasol TK 100; Mintesol; Mintezol; Minzolum; MK 360; MSD 18; Omnizole; Ormogal; Pitrizet; Sanaizol 100; Sistesan; Storite; Syntol M 100; TBZ; Tebuzate; Tecta; Tectab; Tecto; Thiabendole; Thiaben; Thiabenzole; Thibendole; Thibenzol; Thibenzole; Tiabenda; Tiabendazole; Tibimix 20; Triasox; Arbotect; Metasol; E 233.
 Foreign languages: German: Thiabendazol, French: Thiabendazol, Spanish: Tiabendazol.

Health aspects, acute toxicity. In animals thiabendazole is metabolised to 5-hydroxythiabendazole formed by hydroxylation. In humans metabolic products are eliminated with the urine.

LD₅₀ oral of thiabendazole for mice, rats and rabbits moves between 3.1 and 3.8 g/kg body weight. With dosages above 800 mg/kg body weight, growth disturbances occur and the mortality rate increases.

The ADI-level for thiabendazole is set (by the Joint expert committees of the WHO/FAO) to 0–0.3 mg/kg body weight/day.

Antimicrobial action. Thiabendazole is classified among preservatives with a mainly fungistatic action (Arras and Usai, 2001). It is particularly effective in suppressing moulds, especially some penicillium species. Its action against yeast, chiefly debaromyces species, is very weak.

Applications. Thiabendazole is permitted in some countries for the preservation of citrus fruit and bananas up to a maximum quantity of 10 mg/kg and 3 mg/kg respectively. This is its single usage in food technology. In concentrations of 0.1 to 0.45% it may also be added to wax or plastic emulsions or solutions, which are used for treating the fruit surface. Alternatives in this application area, like mild heat treatment or yeast antagonists are under current discussion (Rodov et al., 2000; Usall et al., 2001). Alternative treatments of apples with acetic acid vapour and yeast lead to comparable good results, like thiabendazole processings (Sholberg et al., 2001; Spadaro et al., 2002).

5.10.2.13 Biphenyl [II, 20.7.]

History. Before and after World War II, biphenyl was of major importance in chemical industries in Europe. The main applications are in the area of different organic synthesis, use as a heat transfer agent, as a dyestuff carrier for textiles, and as pharmaceutical flavouring. Since World War II biphenyl has been in use as food preservative due to its ability to inhibit growth of moulds. Its main application field is the usage on peels of citrus fruits (e.g. lemon, orange, lime, grapefruit) where strong regulations limit the amount of substance, which can be added. In the 1950's biphenyl guaranteed the supply with unmoulded citrus fruits in Europe.

Names

CA Index Name: Biphenyl.
 Other Names: Diphenyl; Bibenzene; Xenene; 1,1'-Biphenyl; Phenylbenzene, PHPH; E 230.
 Foreign languages: German: Biphenyl, French: Biphényle, Spanish: Bifenilo.

Health aspects, acute toxicity. LD₅₀ for rats after oral administration: 2400 mg/kg; Skin rabbit LD₅₀: > 5010 mg/kg.

The ADI-level for biphenyl is set (by the Joint expert committees of the WHO/FAO) to 0–0.05 mg/kg body weight/day (temporary).

Antimicrobial action. Biphenyl is highly active against moulds but there is no activity against spores. Additionally there is virtually no action against moulds of alternaria-, sclerotinia- or phytophthora-type. The action of biphenyl can be stopped by addition of excessive amounts of amino acids containing sulfur, like cysteine or methionine. Biphenyl impedes the syntheses of carotinoids and attacks the cell membrane. Moreover, some enzyme systems (e.g. NAD-oxidase) are disturbed.

Applications. Biphenyl is permitted in some countries for the preservation of citrus fruit surfaces with declaration. To protect fruits it is applied by impregnating the wraps or sheets between fruits and/or cardboard paper packaging material (1–5 g biphenyl/m²). Due to its high vapour pressure some biphenyl is frequently found on the citrus skin up to the maximum permissible level (e.g. in the EC of 70 mg/kg fruit). In the past biphenyl was

applied through a dipping bath or in closed rooms by directing vapour directly on the skin of the fruit. Besides prolonged storage a better nutritional value of the treated fruits can be achieved (Toker and Bicici, 1996; Rajpal-Singh and Surinder-Kumar, 1997; Piyush-Verma and Dashora, 2000).

5.10.2.14 *o*-Phenylphenol [II, 7.4.1.]

History. Like biphenyl, *o*-phenylphenol was of major importance in chemical industries in Europe before and after World War II, as intermediate for dyes, rubber and different laboratory uses. In opposition to biphenyl it is not only used to preserve the surface of citrus fruits but also to protect cosmetics and some technical products (e.g. protein based glues or emulsions), and as an active ingredient in disinfectants and cleaning agents, sometimes used in the food industries.

Names

CA Index Name:

Biphenyl-2-ol.

Other Names:

(1,1'-Biphenyl)-2-ol; *o*-Xenol; 2-Hydroxybiphenyl; 2-Phenylphenol; 2-Biphenylol; Dowicide; Nipacide OPP; Preventol O extra; E 231 (Sodiumsalt: E 232).

Foreign languages:

German: *o*-Phenylphenol, French: *o*-Phénylphenol, Spanish: *o*-Fenil-fenol.

Health aspects, acute toxicity. LD₅₀ for rats after peroral administration: 2.5 g/kg body weight, for cats 0.5 g/kg body weight. In higher concentration an irritation of animal skin can be observed. Some allergic reactions against phenylphenol have been observed.

The ADI-level for phenylphenol is set (by the Joint expert committees of the WHO/FAO) to 0–0.02 mg/kg body weight/day (temporary).

Antimicrobial action. Phenylphenol has already an action at low levels between 10–50 ppm (pH 5–8). Its inhibition effect increases with increasing pH and inhibits mainly the growth of moulds. Phenylphenol has in general a broad range of action against moulds, especially if they are found on citrus fruits, but some cases of resistance are described (Holmes et al., 1994).

Applications. The usage of phenylphenol is allowed to protect the surface of citrus fruits and citrus peels for further processing (e.g. into candied lemon or orange peels). The maximum usage level lies between 2 and 70 mg/kg in the EC. The phenylphenol residue must not exceed 10 mg/kg in citrus fruits in the EC. Its usage on banana-surfaces is no longer permitted. The use of phenylphenol is also proposed for a couple of other fruits, like tomatoes (Hall, 1989). For its application fruits are dipped into 0.5–2% solution around pH 12 of phenylphenol and washed afterwards with tap water. Residues on the fruits remained stable but declined e.g. in orange-oil (Papadopoulou, 1991; Johnson et al., 2001). Residues in products made thereof depend on the type of processing (Friar and Reynolds, 1994).

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5.11 Disinfectants and sanitizers

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5.11.1 Introduction

Disinfectants and sanitizers are used not only in the medical facilities, but also in households, livestock husbandry, foods industry, and public utilities such as tattoo studios, pedicure, solarium and swimming pools.

The term “disinfection” was taken over from the medical field, and consists of two parts of Latin origin, the prefix “dis” denoting reversal or separation, and the adjective “infectivus” denoting infectious (capable of producing infection; pertaining to or characterized by the presence of pathogens). The term “to disinfect” came to use during the years of cholera in 1831, and the medical term “disinfection” started to be used with the discovery of pathogenic germs approximately 100 years ago.

The expression “disinfectant” has different definitions in different countries:

- United States of America (EPA) (OECD, 2002): A substance that destroys or eliminates a specific species of infectious or other public health microorganisms, but not necessary bacterial spores.
- European Union (CEN TC 216) (OECD, 2002): A product which is capable of chemical disinfection. Chemical disinfection: The reduction of the number of microorganisms in or on inanimate matrix achieved by action of a product on their structure or metabolism, to a level judged to be appropriate for a specified defined purpose.
- Canadian Definition (OECD, 2002): An antimicrobial agent capable of destroying pathogenic and potentially pathogenic microorganisms on inanimate surfaces. A disinfectant without specified target organisms on the container label is regarded only as a bactericide.
- International (IFH-Guidelines) (IFH, 2002): A chemical agent that under defined conditions is capable of the destruction of microorganisms, but not usually bacterial spores: it does not necessarily kill all microorganisms, but reduces them to a level acceptable for a defined purpose, for example a level which is harmful neither to health nor to the quality of perishable goods.

It is interesting to note that the European and the Canadian definitions of disinfectants are limited to the inanimate surfaces.

The French “Association Française de Normalisation” (AFNOR, 1997) distinguishes between “antiseptic agents” and “disinfectants”: the “antisepsis” describes the elimination or killing of microorganisms and/or inactivation of viruses associated with living tissues, while the term “disinfection” is used in connection with microorganisms or viruses attached to inanimate objects. However, since the time of Lister (1867) the term “antiseptic agents” is reserved for the agents which disinfect wounds.

Currently, the European standards classify the agents for the “hygienic handwash”, “hygienic handrub”, “surgical handwash” and “surgical handrub” to the group of disinfectants and not to the antiseptics.

The way the disinfectants could be classified into different categories according to the method of their use is given in Table 1.

Table 1 Chemical disinfectants – methods of use and objects of disinfection.

	Method	Objects of disinfection
Gases	Ethylene oxide	Heat-sensitive materials for repetitive use (plastic, feathers)
	Triethylene glycol	Rooms
	Formaldehyde vapors	Rooms, mattresses, air
	Ozone, chlorine	Drinking and waste water
Fluids and solids (which could be dissolved or emulgated)		Hands, skin, surfaces, instruments, rooms, excrements
Sprays		Hands, skin, surfaces, instruments, air, rooms

The expression “sanitizers” and/or “cleaners” are used for products which remove dirt or organic material from objects or surfaces, but do not exert bactericidal, sporicidal, virucidal and/or fungicidal activities, and do not necessarily reduce the level of microbial contamination (IFH, 2002).

Examples of product types/use categories for microbicides are given in the following scheme:

- Public health disinfectants and sanitizers for use in/on
 - Hospitals
 - Medical equipment
 - Eating establishments

- Air ducts
- Mortuaries
- Personal health care disinfectants
 - Denture cleaners
 - Disinfection of intact skin (antiseptics and skin cleansers)
- Non public health (private) disinfectants/sanitizers/bacteriostats for use on/in
 - Dust mops
 - Laundries
 - Carpets
 - Bathrooms, kitchens
 - Air purifiers
 - Water beds
- Veterinary area and domestic animal disinfection
- Food/feed area disinfectants for use on/in
 - Agricultural premises and equipment such as beehives, barns, cattle feedlots
 - Industrial food storage and distribution such as egg handling equipment
 - Food processing plants, dairies
- Drinking water disinfectants for use in
 - Human drinking water
 - Animal or poultry drinking water

5.11.2 Historical review

The following data give an overview on the historical development of the physical and chemical disinfection. Many of the disinfecting agents mentioned in this overview had been used much earlier in wood preservatives in the ship building before they started to be used in disinfectants (Thofern, 1982; Block, 2000).

- 450 BC The use of chemical disinfection began during the era of the Persian expansion, when water was stored in silver or copper vessels in order to keep it potable.
- 1677 van Leeuwenhook constructed the first microscope and discovered tiny organisms.
- 1771 Smoke powder consisting of two parts sulfur and one part saltpeter, mixed with juniper berries, myrrh or frankincense, shoots of pine or fir, was invented by the Russians and used during the Volga plague, and was thence called the plague powder. Still in 1830 the procedure was recommended in the Meyer's Konversationslexikon.
- 1774 Scheele discovered chlorine.
- 1775 Pringle described the preservative property of salt and defined the "salt standard" based on the activity of the sea salt.
- 1785 Berthollet used chlorinated water for bleaching.
- 1789 Arab physicians used mercuric chloride for the treatment of wounds.
- 1798 Alcock started the production of bleaching powder.
- 1818 Thenard described hydrogen peroxide.
- 1821 Labarague recommended hypochlorite as an antiseptic.
- 1825 Isfordink recommended cleaning of impure water by boiling.
- 1827 Bleaching powder was used as deodorant and disinfectant.
- 1830 Iodine tincture was introduced in the American pharmacopoeia.
- 1831 Hueter introduced treating of wounds with a hypochlorite solution.
- 1834 Runge succeeded in isolating of phenol (carbolic acid) from coal tar.
- 1835 Disinfection decree was enacted in Prussia.
- 1840 Schönbein discovered ozone.
- 1843 Lefevre introduced the use of chlorinated water.
- 1847 Semmelweis introduced in Vienna the disinfection of hands by chlorinated lime.
- 1849 Frankland synthesized the first organic tin compound.
- 1851 Hofmann discovered the quaternary ammonium bases.
- 1855 Thorr applied dry heat as disinfection means in cholera quarantines.
- 1860 Chefs and Lister used pure liquid phenol as antiseptic agent in the kitchens and the surgeries.
- 1861 Lemaire recommended phenol as disinfection means.
- 1862 Pasteur introduced sterilization by means of high-pressure boiling.
- 1867 Lister performed experiments with phenol as an antiseptic.
- 1868 Hofmann discovered formaldehyde.

- 1869 Raulin described the antibacterial properties of silver-containing compounds.
- 1869 Schmidt discovered the microbicidal effects of hydrogen peroxide.
- 1874 Buchholtz discovered the effects of ethyl alcohol on the microorganisms.
- 1875 Meyer's Konversationslexikon described Kali Permanganicum (Potassium permanganate) as the drinking-water disinfectant.
- 1877 Downes and Blunt discovered the germicidal effects of the blue components of the light spectrum.
- 1881 Koch, Gaffky and Löffler introduced the steam disinfection.
- 1883 Koch discovered the methods to evaluate the antimicrobial efficacy of disinfectants.
- 1886 Schimmelbusch used superheated steam for sterilization in the Bergmann's clinic in Berlin.
- 1886 Two cases of cholera near Mainz lead Rautenstrauch to a systematic testing of disinfectants. The outcome of the testing was the introduction of the carbolic liquid soap.
- 1887 Kossiakoff reported on the adaptation of bacteria to disinfectants.
- 1888 Fürbringer in Jena introduced a disinfectant composed of 80% alcohol and 0,2% sublimate or 3% phenol.
- 1889 Experiments of Rautenstrauch resulted in the introduction and production of the disinfectant "Lysol".
- 1892 "Lysol" was used as disinfectant during the cholera outbreak in Hamburg.
- 1892 Ohlmüller suggested to use ozone for the disinfection of water.
- 1892 Nägeli introduced the expression "Oligodynamics" to describe the germicidal properties of metal ions.
- 1893 Traugott studied the effects of hydrogen peroxide.
- 1893 Tindal constructed an ozone facility in Leiden to disinfect the Rhine water.
- 1894 Traube suggested that drinking water in Germany should be chlorinated.
- 1895 The gynecologist Ahlfeld introduced alcohol free of additives as a disinfectant.
- 1897 Woodhead in Madstone disinfected a typhoid fever-infected water main with a hypochlorite solution.
- 1897 Cresol liquid soap "Bacillol" came to the market.
- 1898 Blotz suggested using sodium peroxide for the disinfection of water.
- 1900 A patent was granted for the method of producing "Lysoform", a disinfectant made from the Kali soap and formaldehyde.
- 1900 Discovery of peracetic acid.
- 1905 Flügge introduced the distinction between the surgical and the hygienic disinfection of hands.
- 1907 Chloramines were introduced.
- 1908 Grossich introduced tincture of iodine in the surgery.
- 1910 Darnall in the U.S.A. introduced chlorine gas for the disinfection of drinking water.
- 1910 Masson discovered that the acquired resistance of bacteria against disinfectants retreats in time without obvious reasons.
- 1912 Regenstein performed an extensive research concerning the adaptation of bacteria to the disinfectants.
- 1915 Dakin described chlorine-releasing compounds.
- 1916 Jacobs discovered quaternary ammonium compounds and their antibacterial effects.
- 1916 Jacobs discovered the bactericidal effects of the quaternary hexaminium salts.
- 1931 "Baktol" as a phenolic disinfectant came to the market.
- 1935 Domagk introduced quaternary ammonium salts as well disinfecting, wetting and cleansing means.
- 1942 Kunz and Gump were granted a British patent concerning the germicidal effects of tetrachlorophene and hexachlorophene on *Staphylococcus aureus*.
- 1943 Sprowls and Poe described the antibacterial effects of the tin compounds. The following research led to the practical use of organic tin compounds.
- 1944 Schmitz discovered the microbicidal effects of the alkyl-polyamino acetic acids.
- 1944 Traub, Newhall and Fuller reported on the comparative tests with different Hexachlorophene solutions and Hexachlorophene soaps.
- 1949 Herrmann and Preuss reported on the experiments with the amphoteric disinfectants.
- 1949 The antibacterial effects of the peracetic acid were reported.
- 1954 Davies et al. described the antibacterial effects of the biguanidine derivative 1,6-di-4'-chlorophenyl-guanidino-hexane "Hibitane".
- 1963 Pepper and Chandler, as well as Stonehill, Krop und Borick reported on the disinfecting effects of glutardialdehyde.

5.11.3 Application fields

The application fields for disinfectants can be divided into three areas following the normative work of the CEN Technical Committee 216 "Chemical disinfectants and antiseptics" (CEN, 2002):

- Human medicine,
- Food, industrial, domestic, and institutional areas, and
- Veterinary field.

Human medicine

Disinfection and sanitizing are the preventive measures designed to protect patients, personnel and other persons who come in contact with infectious diseases. Application fields of these preventive measures are those areas, rooms or situations to which the disinfecting and sanitizing measures apply. These are, above all, the areas which deal with the treatment of patients, e.g. in hospitals, dental practices, acupunctural treatment, laundries and hospital kitchens which deliver their products directly to the patients. Special attention has to be paid to the large kitchens with their fore- and post-processing areas. There is also the need of intervention in the case of increased occurrence (outbreaks) of certain infectious diseases in schools, kindergartens, geriatric nurseries, nursery homes and health resorts, as well as at work places and/or at home. Other application areas are service enterprises such as the hairdresser stores, manicure, pedicure, and piercing and tattoo studios.

More detailed information about the disinfection measures and methods used in the medical field/human medicine is given in section 5.11.4.

Food, industrial, domestic, and institutional areas

These areas include processing, distribution and retailing of food of animal origin (milk, meat, fish, eggs, and related products, animal feeds, etc.) and vegetable origin (beverages, fruits, vegetables, flour, etc.). (For more details on this scope see chapter 5.10.). Further fields are the industrial areas which deal with packaging materials, biotechnology (yeast, proteins, enzymes), pharmaceuticals, cosmetics and toiletries (see also chapter 5.9.), textiles (see chapter 5.16.), computer industry, etc. In the institutional and the domestic areas, the application fields are catering establishments, public areas and transportation, schools, nurseries, shops, gyms, hotels, offices, dwellings, etc.

These application fields exclude areas and situations where disinfection is medically indicated, and also exclude products used on living tissues except those for the hand hygiene in the above-mentioned areas.

Veterinary field

The veterinary field includes the breeding, husbandry, production, transport and disposal of animals, except their use in the food industry after slaughtering.

5.11.4 Disinfection in the medical field/human medicine

Disinfection measures in the medical field consist of the treatment of

- Hands/skin,
- Surfaces,
- Instruments, and
- Laundry.

Hands/Skin

The need of hand disinfection is known since it was demonstrated by Professor Ignatz Semmelweis in 1848. Insufficient hand disinfection is still the main reason of the transmission of microorganisms in the medical practices, hospitals and the food areas (Rotter, 1990). It depends on the application field as to what disinfection method (and what product) has to be chosen in order to reach the sufficient or the highest possible safety level in preventing the undesired transmission of microorganisms. In the hand hygiene, the spectrum of methods varies between the hygienic hand disinfection, which reduces only the transient microbial flora of the skin, and the surgical hand disinfection, which has to minimize the microbial flora of the skin.

“Hygienic handrub” is thus defined as a post-contamination treatment of the hands, without the use of water, which is directed against the transient microbial flora of the skin. The purpose of the hygienic handrub is the prevention of the transmission of the microbial flora and the protection of the personnel from infections. The purpose of the “hygienic handwash” is the same, but in this method water and an antimicrobial product are used. The main difference between these two methods is that in the hygienic handrub we minimize the risk of both the transmitting of the microorganisms into the environment and the recontamination of the hands with microorganisms present in the tap water.

“Surgical handrub” is the treatment of hands before the surgery, without the use of water, and it is directed against the transient and the resident microbial flora of the skin, in order to prevent the transmission of microorganisms into the surgical wound. “Surgical handwash” includes the use of water and an antimicrobial product (prEN 12054, 2001, see also Table 3.).

“Skin disinfection” is required before medical interventions such as injections, punctures and operations, and it is required in order to reduce the levels of the transient and resident microbial flora of the skin.

Products used for the hand and skin disinfection ought to increase their bactericidal (vegetative phases), fungicidal, and virucidal efficacies. They should deliver their effects more rapidly, and they should exert no toxic effects on the skin. Disinfectant solutions used for the hygienic handrub mainly consist of alcohols (II, 1.)*, halogens (II, 21.2.), phenolics (II, 7.), or PVP-iodine (II, 21.2.12.), and are frequently combined with other ingredients. Products used for the surgical handrub mainly consist of alcohols, such as ethanol 80%, isopropanol 70%, n-propanol 60%, or mixtures of those.

Surfaces

Surface disinfectants in the medical field are used for the disinfection of inanimate surfaces, such as floors, walls and furniture (Simmons, 1985) and other materials which come in a direct or an indirect contact with patients and persons. The areas to which this method of disinfection applies are the hospitals, communal medical facilities and dental institutions, school clinics, kindergartens, nursing homes, work places, and the home (prEN 13713, 1999, see also Table 3.).

The mode of treatment is wiping the surfaces of the fittings, technical devices and the floors with a cloth or a scrubbing brush in order to physically disattach the microorganisms from the surfaces, and to bring the disinfectant to the surfaces.

The so-called “ongoing disinfection” during the nursing and treatment of patients deals with a routinely performed disinfection of those surfaces in the vicinity of the patient which are or might be contaminated with pathogens (e.g. bed, table, chair, children’s toys). Visible and massive contaminations have to be disinfected immediately. Those surfaces which are not close to the patient, such as walls 1,5 m above the floor and the ceiling are not a usual source of dust and microorganisms. An infrequent cleaning of these surfaces is sufficient, provided that there has been no contamination of these surfaces with infectious material.

The “final disinfection” is the disinfection of an area or a room that was used for the nursing or treatment of patients who suffered from infectious diseases. These areas have to be disinfected in a way which completely suppresses the danger of infection of the next patient. The final disinfection reaches all those surfaces and objects in this area, that could have been contaminated with pathogens.

The “room disinfection” is defined as the complete and simultaneous disinfection of all surfaces and the air in a closed room by vaporizing or atomizing an aqueous solution of formaldehyde (II, 1c.) (RKI, April 1986). Because this is a large-scale method, e.g. in Germany a strong indication is required by the German Infection Protection Act (FRG, 2000).

The disinfection of surfaces is directed against all kinds of pathogen and facultative pathogen microorganisms. Moreover, the products used for the surface disinfection should have a good cleaning efficacy, should be well tolerated by the skin, should not be damaging the treated material, should have a good fragrance, etc.

In order to prevent the development of tolerances or resistances of the pathogens to the disinfectants, a regular change of the disinfectants is highly desirable. The active ingredients of surface disinfectants are aldehydes (II, 2.), quaternary ammonium compounds (II, 18.1.), and phenolics (II, 7.). Alcohols can only be used for the disinfection of small areas due to the risk of explosions. The bacterial spores ought to be destroyed by peroxygen compounds, such as the peracetic acid (II, 21.1.).

Instruments for medical use

Surgical instruments, accessories and utensils have to be submerged in disinfectant solutions immediately after their use. They have to remain in the solution until the further preparation. This measure is meant to prevent the infection of the cleaning personnel, and also to prevent the drying of the contamination and the spread of microorganisms. The active solution should have an efficient disinfecting and cleaning activity. The good cleansing ability is necessary for the removal of contamination and exposing of the microorganisms to the disinfecting agents. The first disinfection of the instruments should be followed by mechanical cleaning, and should be finished by an automatic cleaning process and final disinfection. The method of choice for the intermediary cleaning of the instruments is the steam sterilization. The heat-sensitive materials should be treated by chemical means.

*see Part Two – Microbicide Data

Instrument disinfectants should not be toxic for the users, should exhibit good cleaning activity on the surfaces and the lumina of the instruments, and should not be damaging the sensitive parts of the instruments, such as the optical lenses of endoscopes. They should also exhibit a good bactericidal, fungicidal and virucidal efficacy. The efficacy of the disinfectants against mycobacteria (Bansemir *et al.*, 1996; Gebel *et al.*, 2000) and *Helicobacter pylori* (Cronmiller *et al.*, 1999) has been the topic of intensive discussion in the recent years. There has been much concern about the cleaning of endoscopes (Ayliffe, 2000). The active ingredients used in the instrument disinfectants are aldehydes (II, 2.), QACs (II, 18.1.), phenolics (II, 7.), and alcohols (II, 1.). Recently a discussion started concerning the treatment of instruments potentially contaminated with prions. In this special case, very alkaline (pH > 10) product combinations with amines are recommended (RKI, 2002).

Laundry

Laundry that cannot be washed should be disinfected by treatment with steam, formaldehyde-steam (II, 2.1.), and chemical dry disinfection/cleaning.

All the other washable hospital laundry has to be disinfected and washed. According to the German regulations (HVBG, 1997) the laundry has to be divided into three groups:

- Extreme infectious laundry: This includes laundry from epidemic wards and of patients who suffer from small-pox or hemorrhagic fever. It has to be disinfected within the wards, and only after this it is allowed to be washed with the potentially infected laundry.
- Infectious laundry: This includes laundry from infection wards, microbiological laboratories, and the pathology department, and has to be disinfected using methods and products given the RKI-list (RKI, 1997).
- Potentially infected laundry: Any other laundry has to be washed using a disinfectant.

Heat-resistant textile can be disinfected by boiling at approx. 100°C for 30 min. The heat-sensitive laundry has to be disinfected by treatment with chemicals, i.e. by an immersion in chemical disinfectants for up to 12 hours at room temperature, or by washing-disinfection in washing machines which allow the control of the critical parameters (concentration of the washing detergent and the disinfectant, the proportion of laundry to the water level, the temperature, and the contact time). When treating infectious laundry, the textile and the washing water have to be disinfected before the first exchange of the washing water (Alexander *et al.*, 1995; RKI, 2000).

For the chemo-thermal disinfection of the laundry, the washing has to be performed at minimally 40°C, and the active ingredients of the washing detergents are aldehydes (II, 2.), QACs (II, 18.1.), phenolics (II, 7.), and chlorine-releasing agents (II, 21.2.).

5.11.5 Efficacy of disinfectants

Factors affecting the efficacy of disinfectants

The efficacy of disinfectants is affected by various factors, such as the target microorganisms, the environment of the microorganisms, the contaminated object, the microbicidal ingredients, and the mode of application. Three of these five factors – the microorganisms, the environment, and the object – are given by the disinfection task. The other two factors – the microbicide, the mode of application – depend on the disinfection method itself (Spicher, 1996).

The main factor is, naturally, the target microorganism, inasmuch as the microorganisms differ in their sensitivity to disinfectants (Spicher and Peters, 1976; Russell *et al.*, 1986; Kaulfers, 1995; McDonnell and Russell, 1999). Testing the bactericidal efficacy of disinfectants therefore includes one (or more) Gram-positive bacterial species (*Staphylococcus aureus*, *Enterococcus faecium*), and one (or more) Gram-negative species (*Pseudomonas aeruginosa*, *Escherichia coli*). The testing against fungi is performed mainly with *Candida albicans* as the representative of the yeasts, and *Aspergillus niger*, as the representative of the molds. Additional test organisms are available according to the claim of the tested product, i.e. tuberculocidal products are tested with *Mycobacterium terrae* or *Mycobacterium avium*. Another test criterion is the concentration of the microorganisms and their environment. A new topic in the health care is the disinfection of biofilms (Exner *et al.*, 1987; Donlan, 2001; Chapter 6.1.).

Other factors, which affect the efficacy of the disinfectants, are:

- Organic load
- Presence of protective agents (proteins, blood, lipids, carbohydrates, minerals)
- Presence of biofilms
- Temperature
- pH
- Contact time

- Disinfectant concentration
- Humidity
- Inactivation of the microbicide by cleaning agents.
- Mechanical action
- Interaction between the microbiocide and the material (e.g. electrical charging)

Test methods

The evaluation and listing of the effective disinfectants and disinfection procedures were performed till now by appropriate national institutions, e.g. AOAC (AOAC, 2002), DGHM (Gebel *et al.*, 2001), AFNOR (AFNOR, 1997), and the BSI (BSI, 1991).

In 1990, the CEN created the Technical Committee TC 216 for the standardization of methods of testing the activity of chemical disinfectants and antiseptics. The CEN test program for chemical disinfectants and antiseptics includes 3 phases:

- A suspension test (phase 1) for the assessment of the basic bactericidal/bacteriostatic or fungicidal/fungistatic activity of the product.
- Tests under conditions representative of practical use (phase 2). These tests include a quantitative suspension test (phase 2/step 1) as well as other laboratory tests, e.g. surface tests simulating practical conditions, handwash and handrub (phase 2/step 2).
- Field tests under practical conditions (phase 3).

The microbicide effectiveness is thus determined in quantitative tests. This is the only procedure which allows determining the killing kinetics of biocides.

The purpose of the qualitative tests is to determine the relationship between the effective concentrations and the effective exposure times with different microorganisms. The in-vitro tests phase 1 and/or phase 2/step 1 are designed to provide information concerning the microbicidal properties at different concentrations, exposure times and exposure temperatures. Additionally, the killing kinetics of the microbicides is studied in the presence of organic load with e.g. protein or blood. However, the results of these tests alone allow no conclusions on the effectiveness of the product under practical conditions. Moreover the results of the in-vitro tests determine the requirements for further tests.

The tests designed to imitate the standard usage of the microbicides (phase 2/step 2) allow drawing conclusions concerning the effectiveness of a product under the practical conditions. Due to the multiplicity of different factors, a wide variation of results can be expected.

Tables 2 to 8 provide an overview on the current CEN TC 216 standards and pre-standards.

5.11.6 Normative and legislative regulations

The marketing of disinfectants and sanitizers is subject to different regulations in different countries. In the years 1997–1998, OECD has performed a survey in the participating countries within the framework of the OECD

Table 2 Standards and pre-standards of CEN TC 216 – phase 1.

Standard	All areas (phase 1)
EN 1040, 1997	Basic bactericidal activity
EN 1275, 1997	Basic fungicidal activity
EN 14347, 2001	Basic sporicidal activity

Table 3 Standards and pre-standards of CEN TC 216 – Human medicine – phase 2/step 1.

Standard	Human medicine (phase 2/step 1)
prEN 14476	Virucidal suspension test for disinfectants used in human medicine
EN 12054, 2001	Quantitative suspension test for the evaluation of bactericidal activity of products for hygienic and surgical handrub and handwash
prEN 13713, 1999	Surface disinfectants used in human medicine, bactericidal activity
prEN 13727, 2000	Medical instrument disinfection, bactericidal activity
prEN 13624, 1999	Medical instrument disinfection, fungicidal activity
prEN 14348, 2001	Medical instrument disinfection, mycobactericidal activity
WI 216032*	Medical instrument disinfection, sporicidal activity
prEN 13623, 1999	Bactericidal suspension test for water treatment products against <i>Legionella pneumophila</i>

*These work items are in the status of inquiry

Table 4 Standards and pre-standards of CEN TC 216 – Human medicine – phase 2/step 2.

Standard	Human medicine (phase 2/step 2)
EN 1499, 1997	Hygienic handwash
EN 1500, 1997	Hygienic handrub
prEN 12791, 2001	Surgical hand disinfectants
WI 216019*	Surface disinfectants
prEN 14561	Medical instrument disinfection, bactericidal activity
prEN 14562	Medical instrument disinfection, fungicidal activity
prEN 14563	Medical instrument disinfection, mycobactericidal activity
WI 216036*	Medical instrument disinfection, sporicidal activity
WI 216037*	Medical instrument disinfection, virucidal activity

*These work items are in the status of inquiry

Table 5 Standards and pre-standards of CEN TC 216 – Veterinary field – phase 2/step 1.

Standard	Veterinary field (phase 2/step 1)
EN 1656, 2000	Quantitative suspension test for the evaluation of bactericidal activity
EN 1657, 2000	Quantitative suspension test for the evaluation of fungicidal activity
prEN 14204, 2001	Quantitative suspension test for the evaluation of mycobactericidal activity
prEN 14675	Quantitative suspension test for the evaluation of virucidal activity
WI 216040*	Quantitative suspension test for the evaluation of sporicidal activity

*These work items are in the status of inquiry

Table 6 Standards and pre-standards of CEN TC 216 – Veterinary field – phase 2/step 2.

Standard	Veterinary field (phase 2/step 2)
prEN 14349	Quantitative surface test for the evaluation of bactericidal activity - nonporous surfaces - without mechanical action

Table 7 Standards and pre-standards of CEN TC 216 – Food, industrial, domestic and institutional areas – phase 2/step 1.

Standard	Food, industrial, domestic and institutional areas (phase 2/step 1)
EN 1276, 1997	Quantitative suspension test for the evaluation of bactericidal activity
EN 1650, 1998	Quantitative suspension test for the evaluation of fungicidal activity
EN 13704, 2002	Quantitative suspension test for the evaluation of sporicidal activity
EN 13610, 2002	Quantitative suspension test for the evaluation of virucidal activity against bacteriophages

*These work items are in the status of inquiry

Table 8 Standards and pre-standards of CEN TC 216 – Food, industrial, domestic and institutional areas – phase 2/step 2.

Standard	Food, industrial, domestic and institutional areas (phase 2/step 2)
prEN 13697, 2001	Quantitative non-porous surface test (without mechanical action) for the evaluation of bactericidal and/or fungicidal activity

Pesticide Programme, the purpose of which was to establish an overview of the prevailing biocide regulations. The survey dealt also with preservatives/microbicides, anti-fouling products, wood preservatives and structural treatments, microbicides for waste disposal and strip mine sites, products used in aquatic non-food sites (e.g. molluscicides, algicides), and products used for vertebrate and invertebrate pest control.

Eighteen countries and the European Commission (EC) responded to the survey. The response from the EC described the approach to be used within Europe once the Biocidal Products Directive (98/8/EC) (see also chapter 5.) is integrated into the national laws of the EU countries (i.e. by 13 May 2000).

The survey responses showed a great variability among countries with respect to use categories regulated and the laws and government departments involved. There were big differences in the use categories regulated by an approval system. For some countries, one law will cover all use categories, while in others several and sometimes many pieces of legislation are involved. Although in most countries, one ministry or agency is responsible for a particular use category or group of categories, there are a few countries that have very complex systems. These countries may split responsibilities between different ministries and between different agencies within the same ministry for the approval not only of different product types but even for the same product type.

Regulatory procedures appear to be more similar. Most countries assess data on the active ingredient and the formulated end-use product in one procedure as an integrated package, but currently approval is only granted to the product. In a few countries, and in the forthcoming EU system, active ingredients have to be approved first before they can be used in products. Conditions for how a product should be used are usually attached to approvals. Most countries have decision-making criteria for “approval” or “notification” and “classification” and “labeling”. Risk-benefit generally plays a role in decision-making, and all countries indicate that they can deny approval of an active ingredient or end-use product.

‘Notification’ is a system in which a manufacturer/supplier must inform the appropriate national authority about their intention of placing a chemical on the market. Typically, the notifier must submit data for review by the national authority. There is usually a set time for the review period (e.g. 60 days). The national authority may decide on the classification and labeling of the chemical and may recommend/take control measures.

‘Approval’ (or registration) is a system in which a chemical can only be placed on the market when the regulatory authority has given its permission. Again, the national authority will review data submitted by the manufacturer/supplier but under this system the authorities have much more control over the use of the substance. Formalized data requirements generally exist for use categories regulated by an approval system.

Efficacy data are always required but not necessarily for all use categories regulated. Demonstrated efficacy is often important when deciding whether to approve a product.

The approved use of a particular product and the target pest should usually be specified on the label. All countries require that products be labeled for hazard, and most also require the labels to carry warnings or restrictions based on risk.

Environmental exposure assessment of biocides appears to be less systematically performed than does human exposure assessment. Some countries perform environmental exposure assessments for all use categories regulated, while others perform them on a case-by-case basis. All countries recognize the importance of use data in their assessment procedures, but they also recognize that collecting accurate information is difficult. Environmental monitoring data are not normally required, but are used when available.

Relevant laws

For some countries, one law covers all biocide use categories that they regulate (Ireland, Netherlands, UK, and Australia for biocides approved), while in others, several and sometimes many (Hungary) pieces of legislation are involved.

The type of laws covering disinfectants/sanitizers (OECD uses categories 1–6) varies considerably from country to country. Countries such as the Netherlands, Portugal and USA regulate the majority of these products using pesticide legislation. Canada, Germany and Greece regulate them as medicines/drugs, while New Zealand and UK use chemicals/toxic substances regulations. For other countries (e.g. Australia, Belgium, Denmark, Sweden), a mixture of pesticide and non-pesticide legislation is used, depending on the use category.

Regulatory procedures for active ingredients, end-use products, new and old biocides

Most countries assess data on the active ingredient(s) and the formulated end-use product in one procedure as an integrated package, but approval is usually only granted to the end-use product.

In other countries, and in the forthcoming EU system, active ingredients have to be approved first (and placed on a ‘positive list’ as in the EU) before they can be used in products.

In Canada, there is a different approach to the treatment of active ingredients between different use categories. For all disinfectants regulated under the Food and Drugs Act, once an active ingredient is approved, all sources of that active are considered to be acceptable for use. However, for biocides regulated under the Pest Control Act, individual registration of all sources of an active ingredient is required, i.e. there is no generic registration process.

The approach to approval in Sweden should be noted. In Sweden, when an application for approval of a biocide is received, the need for the product is first reviewed. If there is considered to be no need, approval will not be given. If there is a need, then the human health and environmental risks of the active ingredient(s) will be evaluated. A risk-benefit analysis is then performed, and depending on the outcome, the product might get approval.

Generally the regulatory procedures for ‘new’ and ‘old’ biocides are the same. However, data requirements may differ slightly or even a lot. If the active ingredient(s) or the use category of the biocide product is new, a full dossier of information is usually required from industry. For products based on existing active ingredients, new data may not be required if access to data previously reviewed is possible and the approval procedure is generally very straightforward.

Harmonized CEN standards

Harmonized CEN Standards are standards based on the guidelines of the Council of Europe. The preparation of these standards was requested by the European Commission from the appropriate CEN committees. These standards are drawn up by the European standards institutions CEN/CENELEC together with the national standards institutions, and are published by the European Commission in the “Official Journal of the European Communities” as harmonized standards. These standards are then converted into national standards, and are denominated as the “Harmonized Standards” (e.g. DIN-EN).

These standards specify the legal requirements placed on the products, procedures (e.g. clinical testing, surveillance), institutions and persons. However, the compliance with the standards is of a voluntary character. For instance, §6 MPG says: “The adherence to the provisions of this act is expected for those medical products which correspond to the harmonized standards concerning the appropriate medical products.”

The harmonized standards are therefore not legally binding, and have only a voluntary character for the user. They are based on presumptions which may be vitiated. However, the manufacturers who deviate from the rules set by these standards have to be ready to prove that safety level of their products is as high as it would have been, had the rules set by the harmonized standards been honored.

ISO-Standards

The ISO-Standards are produced by the commissions of the “International Organization for Standardization” for the worldwide use. They also have a voluntary character for the users but may also be referred to by courts in order to assess the safety level of the products or the processes in question.

USA – EPA and FDA regulations (see also Chapter 5.1.)

Antimicrobial agents used on inanimate objects and surfaces are regulated as pesticides by the Environmental Protection Agency (EPA). EPA registers and regulates antimicrobial pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). To obtain registration, manufacturers of antimicrobial products must meet the basic standards:

The product may not cause unreasonable adverse effects to human health or the environment, and the product labeling and composition may comply with the requirements of FIFRA. Moreover, manufacturers are required to submit to EPA detailed and specific information concerning the chemical composition of their product; effectiveness data to document their claims against specific microorganisms and to support the directions for use provided in labeling; labeling that reflects the required elements for safe and effective use; and toxicology data to document any hazards associated with use of the product.

Products intended for the control of fungi, bacteria, viruses or other microorganisms in or on living humans or animals are considered drugs, not pesticides, and are therefore regulated by the U. S. Food and Drug Administration (FDA).

The Federal Food, Drug, and Cosmetic Act (FDCA) is the basic food and drug law of the U.S. The term “drug” in this act is defined as

- Articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them
- Articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals
- Articles (other than food) intended to affect the structure or any function of the body of man or other animals.

Summary

Disinfectants and sanitizers are used in the public and personnel health care, as well as in the non-public (private) health care, the veterinary and domestic animal areas, the food and feed areas, and in the drinking water treatment.

Disinfectants are generally defined as chemical agents that under defined conditions are capable of destroying or removing microorganisms, but not usually bacterial spores. They do not necessarily kill all microorganisms, but reduce them to the levels which are acceptable for the defined purpose.

Sanitizers are products used for the removal of soil or organic material from objects or surfaces. These products do not exert bactericidal, sporicidal, virucidal and/or fungicidal activities, and do not necessarily reduce the level of microbial contamination.

Testing the efficacy of disinfectants is influenced by a variety of factors, such as the target organisms, the environment of the microorganisms, the contaminated object, the microbicidal ingredients, and the mode of

application. The evaluation and listing of the effective disinfectants and disinfection procedures are still performed by appropriate national institutions, but a standardizing effort is in progress, e.g. the CEN Technical Committee 216 is currently preparing standards for the evaluation of the efficacy of disinfectants and antiseptics.

The marketing of disinfectants and sanitizers is subject to different regulations in different countries. The responses to the survey on the prevailing biocide regulations performed by the OECD Pesticide Programme in the years 1997–1998 revealed a great variability among the participating countries with respect to the regulated use-categories, and the involved laws and government departments. Most countries use a single procedure to assess the data on the active ingredient and the formulated end-use product. Information on the efficacy is always required, even though not necessarily for all of the regulated use-categories.

Harmonized CEN standards specify the legal requirements placed on the products, procedures, institutions and persons. The compliance with the standards is – as it is with the ISO-Standards – of a voluntary character, but they may also be referred to by courts in order to assess the safety level of the products or the processes in question.

In the United States, the antimicrobial agents used on inanimate objects and surfaces are registered and regulated as pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) by the Environmental Protection Agency (EPA). Products intended for the control of fungi, bacteria, viruses or other microorganisms in or on living humans or animals are considered drugs and are therefore regulated by the U.S. Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act (FDCA).

Acronyms and Abbreviations

AFNOR	Association Française de Normalisation
AOAC	Association of Official Analytical Chemists
BSI	British Standards Institute
CEN	Centre Européen de Normalisation
CENELEC	Centre Européen de Normalisation Electrotechnique
DGHM	Deutsche Gesellschaft für Hygiene und Mikrobiologie
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
HICPAC	Healthcare Infection Control Practices Advisory Committee
IFH	International Scientific Forum on Home Hygiene
PVP	Polyvinylpyrrolidon
RKI	Robert Koch-Institute
QAC	Quaternary Ammonium Compound
TC	Technical Committee
WI	Work Item

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5.12 Microbicide applications in the leather industry

C. HAUBER

5.12.1 Introduction

The basic raw materials for the production of leather are hides and skins. Tanning provides a long lasting preservation to the easily degradable proteins of the skin. In general the natural structure of the skin is maintained along the chemical and mechanical processes from the raw material to the finished leather. Before hides or skins are turned into leather there are several states for an attack of microorganisms so that they may be damaged or destroyed. The effective protection of this sensitive material in raw and semi-processed state by chemical preservatives became increasingly important. In recent years the safe use of microbicides and their ecological properties play an important role.

5.12.1.1 Historical development

Processes for converting animal hides and skins into leather have been known to man for at least 100 000 years. The Stone age people used smoke, oils and fat to preserve skins from putrefaction and to use them for clothing. Certainly they made the experience that hides and skins in a dried state lose their original softness and flexibility but they were stabilised. Later, it was found that the bark and fruit of certain plants contain very effective tannins suited for tanning in pits. Vegetable tanning agents extracted with water from oak bark, chestnut, wattle and other have been used until today. With the progress of the chemical sciences it became possible to find out the chemical structure of the vegetable tanning agents and to prepare similar substances, termed syntans. In the Middle Ages tawing with alum salts came to predominate besides vegetable tanning.

A milestone in manufacturing leather was the discovery of the tanning properties of trivalent chrome salts like chrome sulfate at the end of the 19 century. All the tanning agents have been used so that vegetable, synthetic and mineral tanning can be distinguished.

Tanning has been carried out in pits but currently it is carried out mainly in moving vessels, i.e. drums. Movement supports the tanning process and shortens the time necessary.

The predominant tanning technique in the world is chrome tannage (about 85%). As a multi-purpose material chrome tanned leather can be transferred to leather with different properties (to different leather types) by further processing. However, a significant quantity of vegetable tanned leather is manufactured, too. Part-tanned chrome-free – often termed “wet white” – leather is also produced, in small but growing quantity.

Leather can be used for numerous purposes, e.g. for the manufacture of shoes, bags, garment, furniture and car upholstery. The different requirements to those leathers can be met by choosing the appropriate raw material (hides or skins from cattle, sheep, goat, horse, buffalo, kangaroo but reptiles fishes and birds, too) and by modifying the leather producing processes. The growing demand for special types of leather resulted in more and more sophisticated processes and requires special skills, tools and machines. Along to this development the size of tanneries has grown. The raw material needed for a continuous production cannot be purchased around the enterprises only but must be often shipped over a long distance, in many cases worldwide. That means long-term storage and the need for preservation of hides and skins to avoid losses through hide deterioration.

In some situations hides are directly conveyed from the slaughter house to the tannery for conversion into the tanned semi-processed or ready-for-use leather. In recent years specialisation between tanneries is observed. This results in a considerable movement of hides and skins in semi-processed state. There are enterprises in which hides are prepared for tanning by chemical measures and where tanning takes place. Elsewhere the semi-processed material is used for producing the desired leather type. If necessary or desired the surface of the leather is being treated, finished. Through transport, delivery and longer periods of storage the semi-processed wet leather can be damaged by micro-organisms. The most common forms of partially produced leather are in the wet blue (chrome tanned) or wet white (chrome-free, mainly aldehyde tanned) and the crust (dry, perhaps dyed) condition.

5.12.1.2 The structure of the skin

The technology applied in leather manufacture builds on the characteristics of the raw material, mainly hides and skins. The skin is made up of several layers: the grain, the corium and the flesh (Figure 1). The grain is covered by an outer layer known as epidermis and the hair. Like the epidermis the hair is made up of the protein keratin. The hair follicles in which the hair is embedded reach into the junction between grain and corium.

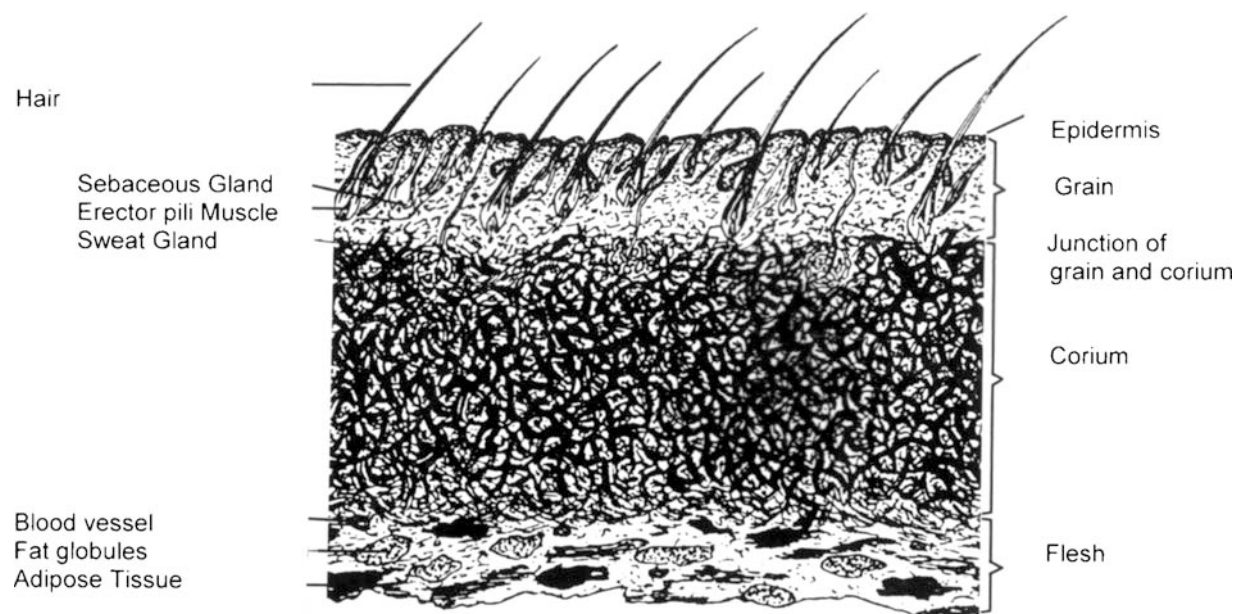


Figure 1 The structure of bovine hide.

The grain contains besides the hair follicles glands, veins and special muscles. The basic substance of the grain is an interwoven fibrous tissue consisting of the protein collagen. The fibres are very fine and especially the outer layer can be damaged mechanically or by microorganisms easily (Herfeld, 1990).

The corium is a relatively thick, the grain supporting tissue. It consists of strongly interwoven, coarse fibres made from collagen, fat and veins.

Due to the chemical and mechanical properties only collagen can be converted to leather. That means leather is made from the grain layer and the corium.

With all types of leather, except fur or wool skins, the epidermis and the hair are removed chemically in the first steps of the leather making process. Flesh tissue is mechanically removed by special machines prior to the tanning.

After slaughter the hide is separated from the carcass by cuts made in the skin and by mechanical pulling or hand flay. After flaying the hide is not protected any more against putrefaction. Decomposition or bacterial attack of the hide readily occurs. Due to residues of blood and dung attached to the hide it is an excellent breeding ground for micro-organisms.

5.12.2 Leather manufacture

The leather making operations can be divided into three major groups:

- the beamhouse processes
- the tanning and
- wet (post-tanning) and dry finishing.

During several beamhouse processes the hide or skin is being prepared for tanning. The first step is soaking the hides in water with the aim to rehydrate the skin and to remove salt, blood, dung and dirt and natural grease from the hide. Detergents, alkali and special enzymes can be added to the soak. Soaking time can last from a few hours to several days.

Soaking is followed by the liming process. Lime and sodium sulfide and hydrogensulfide are the mainly used chemicals for this step. The objective of this process is to remove the epidermis and the hair. The protein keratin is broken down chemically. Another objective is to open up the structure of the leather building collagen fibres. Non-structured proteins and other substances which would hinder the penetration of tanning and other agents are also removed. The pH of the liming float is strong alkaline, above 12. During liming the hide or skin is converted into pelt. Fleshing to remove the flesh and splitting to regulate the thickness of the material and to obtain the most valuable part termed grain layer or grain split is normally done after liming.

The next steps often carried out together are deliming and bating of the pelt. The pH is decreased to about 8 by addition of weak acidic inorganic or organic salts or acids. Residual lime is removed from the pelt. Bating is performed with enzymes like trypsin or bacterial proteases to clean the pelt, to remove degraded protein residues.

Almost all tannages require the skin to be in acid state for avoiding a too rapid fixation of the tanning agent on the surface. In a process called pickling generally sulfuric and formic acid together with common salt are used to reach the level of acidity required for the tannage.

The tanning agent is under this condition able to penetrate into the skin. Tanning is not only penetration of the tanning agent into the skin but means reaction with reactive groups of the collagen to be integrated into a network of fibres and to replace the sensitive hydrogen-bonds of the original protein. Chrome tanning is based on chromium sulfate with a determined basicity. After penetration of the chromium complexes the tanning system is made less acidic by addition of basification agents like magnesium oxide or sodium carbonate to improve the fixation of chromium to collagen. At the end of the tanning process the pelt is converted into a semi-processed leather, the so-called wet blue. Chrome free tanning results in wet white. Through mechanical and further chemical treatment the desired leather type can be produced from the semi-processed leather.

Wet-finishing (or post-tanning) and dry finishing is often carried out in specialised factories. According to the wishes of the consumer typical properties and fashionable effects are given to the leather. In the re-tanning therefore syntans, resins, polymers, vegetable and mineral tanning agents are used. Dyestuffs and fatliquors are added for the desired colour and softness.

In the finishing process the surface of the leather is treated to obtain special effects and to protect it against mechanical damages, to make it more user-friendly. In the finishing polymer binders, dyestuffs, pigments, waxes and others are sprayed or applied to the leather surface.

5.12.2.1 Sensitive process steps

Beamhouse processes, tanning and post-tanning processes are carried out in water. The pH value of the aqueous medium used reaches from strong acidic through weak alkaline to strong alkaline. Depending on the pH, supply of nutrients and water content micro-organisms can grow and cause damage in several processes or states of the product.

The fresh hide or skin is extremely sensitive to damage causing bacteria which benefit from the high water content (about 70%) of such substrates, from pH values moving between neutral and weakly alkaline and from an ambient temperature stimulating bacterial activity. After slaughter the flayed hide keeps its temperature for a relatively long time and due to blood and dung presents a good breeding ground. In soaking similar conditions are provided to bacteria.

Processes with extremely high (liming) or low (pickling) pH values do not favour to the growth of micro-organisms. However, the semi-processed states like wet blue or wet white provide due to their water content (about 40%) and their pH (about 4,0) an ideal breeding ground for mould and yeast.

Finished ready-for-use leather, vegetable tanned leather and crust may have a different water content depending on the environment. A water content below 12–15% inhibits the proliferation of micro-organisms. However, sporogenic species may have released spores which withstand unfavourable conditions, e.g. great heat and dryness, but will germinate to a metabolic active state again as soon as favourable conditions appear.

5.12.2.2 Damages caused by micro-organisms

Bacteria, yeasts and mould secrete enzymes able to degrade macromolecules like proteins, fats and carbohydrates to smaller units that can be absorbed through the cell membranes and serve as nutrients. Stains, dyeing and finishing irregularities are the results of microbial activity.

Bacteria mainly cause putrefaction. The first sign of this is bad smell, later on hairslip, due to the bacterial attack of the hair follicle. Putrefaction may result in considerable loss of substance, loose grain and holes (Figure 2).

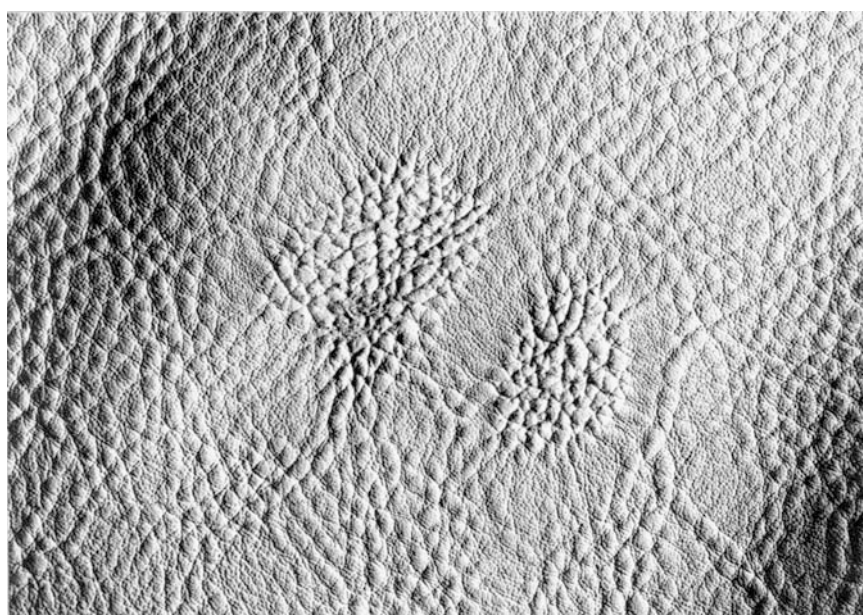
The following bacteria were found in hides and in soaking (Gattner et al. 1988; Rother, 1995):

Bacillus subtilis
Escherichia coli
Proteus vulgaris
Pseudomonas aeruginosa
Aerobacter
Micrococcus
Staphylococcus aureus

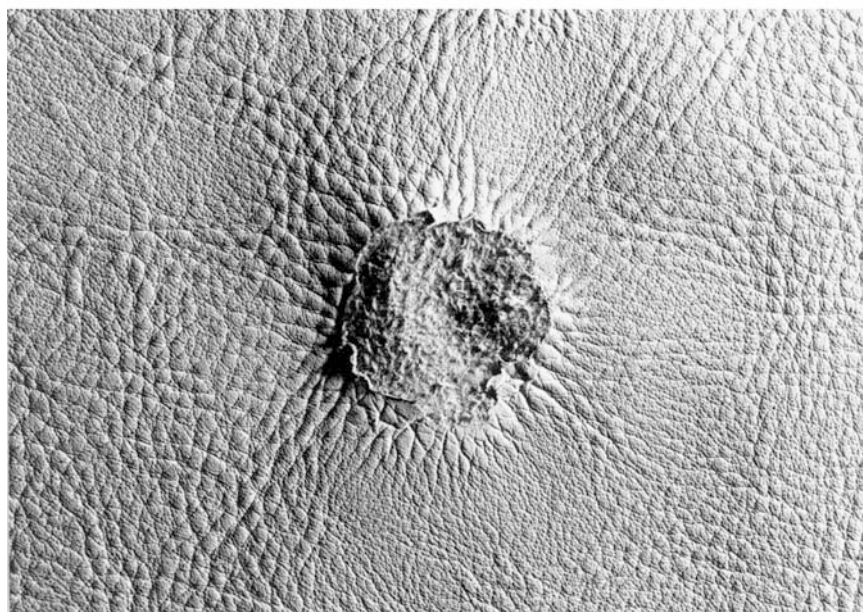
Semi-processed leathers like wet blue and wet white are due to their water content and acidity extremely sensitive to attack by mould and yeast but not by bacteria. If long-term storage under warm conditions is necessary mould may grow on the leather surface (Figure 3) that results in stains. In case of yeast (Figure 4) the stains are much smaller than those caused by mould. Because of the dyeing irregularities the dyed, even the finished leather is damaged. Mould are able to crack fatmolecules so that a kind of fatty spues develop on the surface.

The following mould and yeasts may attack leather (Gattner et al., 1988; Rother, 1995; Birbir et al., 1996):

Mould	Yeast
<i>Aspergillus niger</i>	<i>Candida albicans</i>
<i>Mucor spec.</i>	<i>Torula rubra</i>
<i>Paecilomyces spec.</i>	<i>Saccaromyces cerevisiae</i>
<i>Penicillium funiculosum</i>	<i>Rhodotorula spec.</i>
<i>Trichoderma viride</i>	
<i>Chaetomium globosum</i>	
<i>Aureobasidium pullulans</i>	
<i>Rhizopus stolonifer</i>	
<i>Cladosporium spec.</i>	
<i>Fusarium spec.</i>	



(a)



(b)

Figure 2 Putrefaction caused by bacteria in crust (a + b).

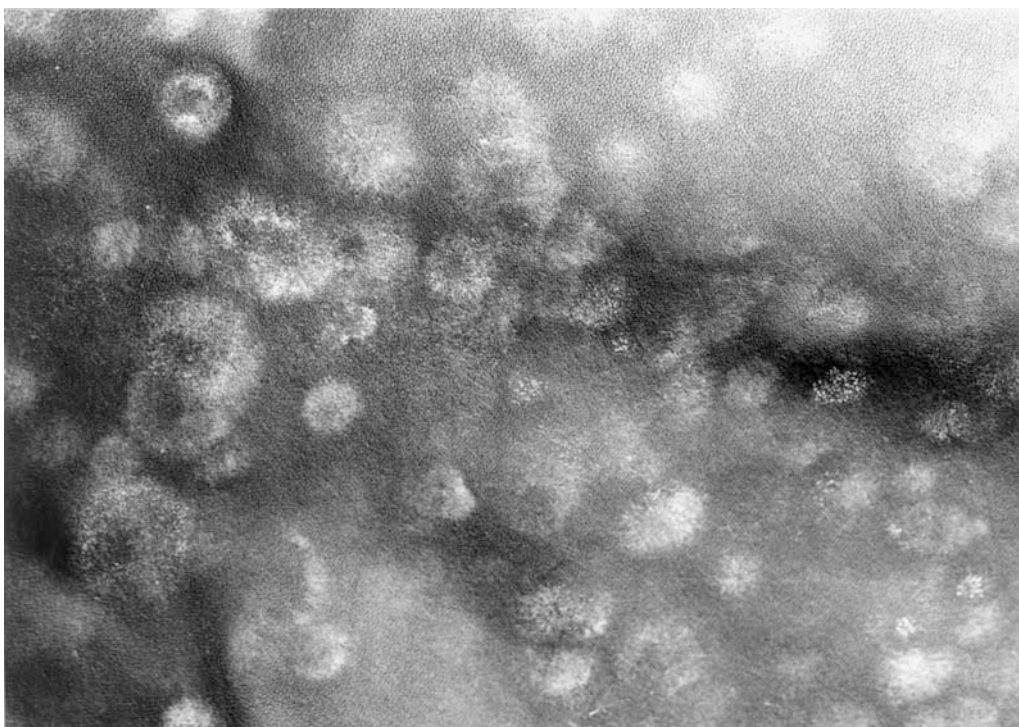


Figure 3 Damage on wet blue caused by mould.

5.12.2.3 *Preservation of the raw hide*

Measures must be taken for a successful preservation and protection of the raw hide and semi-processed states produced from it. The raw hide is the most expensive factor in the whole leather production. If it is not possible to process hides immediately after flaying or to chill them (Adzet, 1997) by direct application of ice to the fresh hide (short term preservation) they can be preserved by salting. The hides are covered with salt and stored in piles. The salt removes water from the skin which drains as brine. By this partial drying of the hide bacterial activity is inhibited, as there is no more unrestrained water left for proliferation of bacteria. In USA brining is preferred that means treating the hides in a concentrated salt solution. The disadvantage of salting is a high pollution load of dissolved solids in the effluent.

Any delay before preservation increases the risk of bacterial damage especially to the sensitive grain layer. Due to special prescriptions for slaughter houses bactericides are not normally used.

Short term preservation of the soak by bactericides may be necessary to avoid damages of the hide and later on of the leather. Requirements on the bactericides differ from those on fungicides. Bactericides need to be effective in a neutral to weak alkaline medium only for a short period of time (1–2 days). They must not have an adverse effect on the hide or the activated sludge in effluent treatment plants.

One of the most frequently used active agents, normally applied at an addition rate of 0,03–0,1% on hide weight is didecyldimethylammonium-chloride [II, 18.1.4.], a quaternary ammonium salt which effects rapidly, is of low toxicity and bio-degradable.

Other microbicides the activity spectrum of which covers not only bacteria, but especially fungi, too, are used as well for soak preservation. Examples are:

sodium dimethyldithiocarbamate [II, 11.11.1.]

N-hydroxymethyl-N-methyldithiocarbamate [II, 3.4.11.]

tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione [II, 3.3.25.]

and 2-thiocyanomethylthiobenzothiazole (TCMTB) [II, 15.11.]

Addition rates move between 0,02–0,1% on hide weight.

The usual dilution of tannery effluents brings the concentration of the active ingredients below the figure which would affect the activated sludge system. The residues are mostly destroyed under the reducing conditions prevailing in tannery effluents prior to their biological treatment.

5.12.2.4 *Preservation of semi-processed leather*

Fungi prefer an environment of weak acid to neutral pH. To provide them with ideal growth conditions substrates need to contain 12–15% water only. The optimum temperature is about 25°C. To protect partially

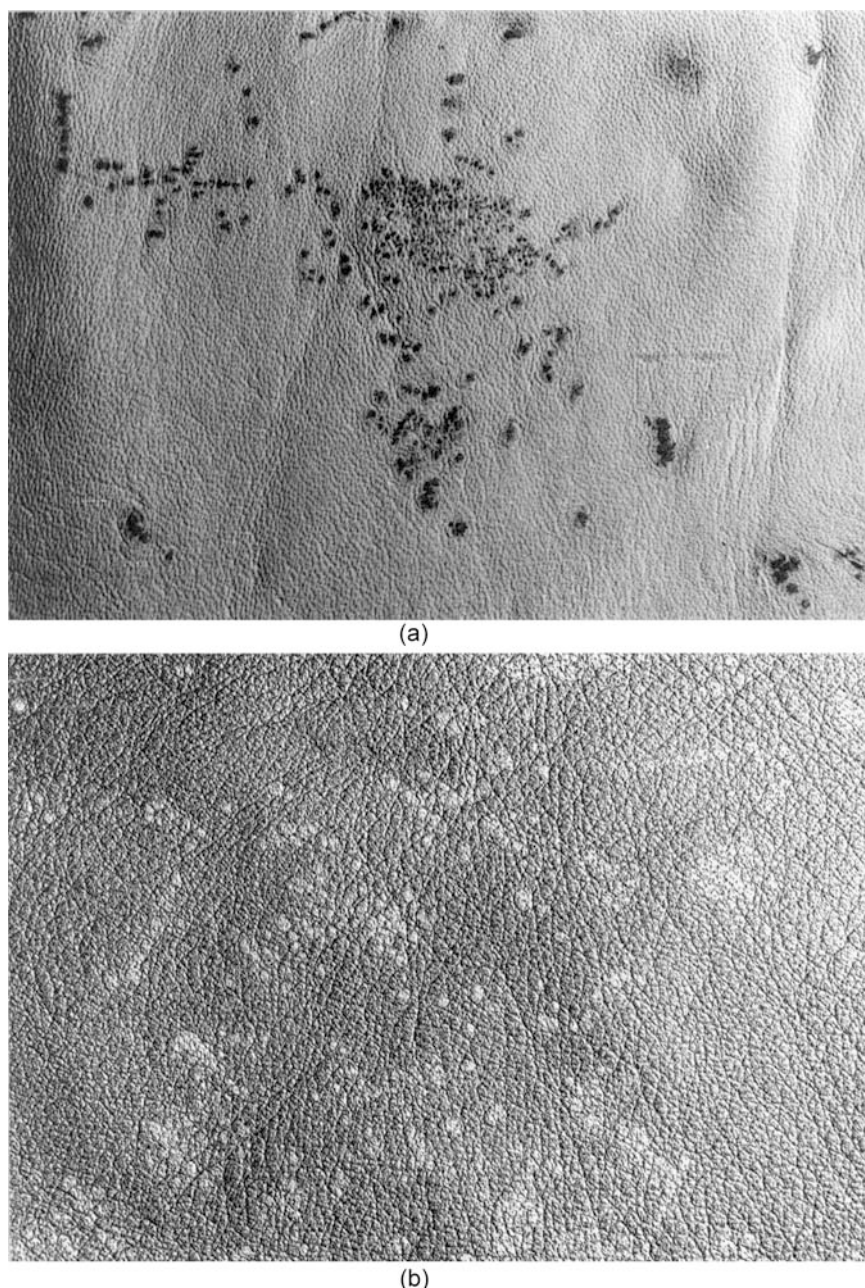


Figure 4 Damage on wet blue caused by yeast (a + b).

processed hides from mould growth fungicides are used suited to guarantee long-term preservation (several months) after application by preference in the tanning liquor.

The fungicide must exhibit activity to a wide range of fungi, should be stable in acid media and not interfere with chemicals used for processing leather. Fungicides causing discoloration, staining or other damages to the leather are not acceptable. On wet blues the antifungal activity should persist during the whole storage period (3–12 months). Suitable fungicides present chemical and physical properties (solubility, substantivity) which enable them to penetrate the leather and to resist to leaching in subsequent washing processes, so that it is always available and active also on the leather surface. It stands to reason that the fungicides show a favourable toxicity and ecotoxicity profile.

Several years ago the predominant fungicides used in the leather industry based mainly on chlorinated phenols [II, 7.5.]. Since the withdrawal of pentachlorophenol (PCP) [II, 7.5.4.] because of the danger of dioxine content alternative products are offered. However, there are other phenol derivates, such as p-chloro-m-cresol (PCMC), [II, 7.3.1.] and ortho-phenylphenol (OPP), [II, 7.4.1.], which meet the requirements now expected of such microbicides very satisfactorily. Both fungicides are also used in vegetable tannage and mainly combined with each other. Alternatives to PCP presenting low toxicity and ecotoxicity data are also found among heterocyclic N,S [II, 15.] compounds, although they are less effective than PCP.

The most important and best known fungicides for use in the leather industry are (Lindner, 1998):

2-thiocyanatomethylthiobenzothiazole (TCMTB), [II, 15.11.]

2-n-octyl-4-isothiazolin-3-one (OIT), [II, 15.4.]

4-chloro-3-methylphenol (PCMC), [II, 7.3.1.]

ortho-phenylphenol (OPP), [II, 7.4.1.]

2-benzimidazolyl-methylcarbamate (BCM), [II, 11.4.]

1,2-benzisothiazolin-3-one (BIT), [II, 15.6.]

The choice of a fungicide type is influenced by a number of factors. Some of them are highly hydrophobic and therefore are adsorbed rapidly by the hide on contact. The uptake and distribution can be uneven and may influence the level of protection of hides within a batch. The addition of the products under practical conditions should be done after 1:10 or 1:20 dilution into the rotating drum to make sure an even distribution in the float.

In some cases the combination of several fungicides is recommended to extend the effect against fungal species. BIT for instance shows gaps in its spectrum of effectiveness and is therefore often applied together with other heterocyclic N,S compounds, e.g. OIT and TCMTB (Muthusubramanian et al., 1998), microbicides which are more sensitive to chemical degradation than BIT. As these microbicides are applied in very low concentrations, even minor quantities of disturbing compounds might cause inactivation of the active ingredients.

The fungicide dosage should be added in several rates. Those hides at the side of the drum at which the fungicide is added are more likely to receive a higher dose of the fungicide than those at the opposite side of the vessel (Stosic et al., 1993).

Time of application is a very important factor which improves the penetration of the fungicide into the wet blue. Usually one half to two third of the addition rate is given to the tanning liquor after the tanning agents had been added, the rest is added prior to the end of the tanning process. Comparing the penetration of TCMTB, OIT and PCMC/OPP into wet blue it was found that addition of TCMTB should be carried out in pickling or at the beginning of the tanning process. The most favourable application time for PCMC/OPP proved to be the time after adding basification agents. The effectiveness of OIT was less when the active was added after the chrome tanning agent that means addition has to be carried out in pickling or at the very beginning of the chrome tanning (Hauber and Germann, 1996; Hauber and Germann, 1997). For the production of wet white the procedure is the same in general but details are not investigated by now.

The distribution of fungicides in the cross section of a leather between grain, middle and flesh layer is not equal. In general the grain split contains 60–70%, the middle 10–20% and the flesh split 25–30% of the absorbed fungicide (Hauber and Germann, 1996; Hauber and Germann, 1997).

For an effective protection in case of long-term storage wet blue treated with fungicides should contain of TCMTB 250–300 ppm, of OIT 100–150 ppm, of PCMC/OPP in combination 580 resp. 280 ppm (Hauber and Germann, 1996; Hauber and Germann, 1997).

If the fungicides are used according to the recommendations of producers and to the general rules of applying fungicides, no effect on the processes in effluent treatment plants is expected. In some cases resistance to microbicides is discussed (King et al., 2001).

Using PCMC containing fungicides AOX values (adsorbable aorganic halogens) may occur in tannery effluent which may pose a problem (AOX in the effluent is limited in many countries).

The fungicide content of wet blue is determined analytically after extraction with ethyl acetate and clean-up of the extract according to a method for HPLC (TEGEWA, 1996). Certain microbiological tests can supply reliable indications for the performance in practice. For example, samples of tanned hide treated with a fungicide can be tested in an environmental chamber. The procedure is similar to the manner in which leather is inoculated and subsequently disfigured by fungi. An agar plate test is based on the leaching rate of the fungicide and relies on the size of a zone of inhibition of test fungi spread on the surface of the agar plate (TEGEWA). The availability of new products is influenced by the European Biocidal Product Directive (BPD) which passed European Parliament in January 1998 (Neuber, 1998). Under this directive, manufacturers of biocides will have to obtain authorisation from each EU-member state before selling their products. See chapter 4.2. The focus of development must be to optimise the use of available preservatives by taking into account their chemical and biological properties.

Acknowledgments

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5.13 Microbial degradation of plastics

P.J. DYLINGOWSKI and R.G. HAMEL

Plastic materials generally are considered non-degradable, yet many materials do suffer from microbial degradation while in service. Signs of degradation include discoloration, odor, or loss of physical properties. The most widely preserved polymer is flexible polyvinyl chloride (PVC), used in swimming pool, pond and ditch liners, as well as shower curtains, upholstery, outdoor patio furniture, roofing (single ply), marine tops, refrigerator gaskets and automotive side molding. Polyolefins and polyurethanes also require preservation in certain applications. The key end-uses requiring preservatives for polyethylene, polypropylene and their associated alloys include mop buckets, roofing, carpet fibers, cutting boards, and the like. Polyurethane (PU) requires preservation when used in shoe soles, shoe uppers, as well as in expanded foam for carpet underlay and padding for furniture cushions and mattresses.

5.13.1 History of plastics and biocides for plastics

The development of synthetic polymeric materials (Table 1) began in the mid-nineteenth century as an effort to find alternatives to materials that were either very expensive or in short supply. The early synthetic materials were chemical modifications of naturally occurring cellulosic polymers. The development of fully synthetic polymers did not occur until the early twentieth century, with fully cross-linked thermoset materials.

Throughout the twentieth century, the pace of innovation developed along with the growth of the petrochemical industry, and scientists developed many new plastics.

Initially there was little recognition of issues related to microbial degradation of plastics. The first antimicrobials used in plastics, in fact, were simply the same materials that were being used to protect natural products from microbiological attack. Some of the difficulties encountered early in the development of antimicrobial treatments for plastics are described in an early patent on the subject (Howard, 1949). The plastics industry was less than eager to take on the challenges of treating plastics with antimicrobials and the commercial introduction of antimicrobial treated plastic was not well documented in the literature. One of the first cited requirements for treated plastic articles came from the U.S. military in the early 1950's (Rei et al. 1992). An early review of the literature on antimicrobials used in synthetic materials (Turner, 1967) included a short selective list of common fungicides for plastics available at the time: N-(trichloromethylthio)-4-4-cyclohexene-1, 2-dicarboximide (Captan[®]) [II, 16.3.]*, copper naphthenate [II, 8.1.12.a.], copper-8-quinolinolate [II, 13.3a.], organomercurial [II, 19.2.], pentachlorophenol [II, 7.5.4.], phenyl mercuric formate, and tetramethylthiuram disulphide [II, 11.13.1.]. The suggested use levels for these materials in plastics ranged from 0.25% to 5%. By today's standards, this is a high use level for an antimicrobial additive. The other striking feature of Turner's list is that it includes several materials with unfavorable human toxicological profiles and potentially harmful environmental effects. Several of these antimicrobial agents, such as organomercurial, tetramethylthiuram disulphide, and pentachlorophenol, are no longer used in plastics because of their possible effects on people and the environment.

Table 1 Timeline for synthetic polymer development

1869	Cellulose Nitrate
1889	Rayon
1910	Phenol-formaldehyde
1927	Cellulose acetate
1931	Poly vinyl chloride
1931	Poly vinyl chloride, Poly vinyl acetate copolymer
1933	Polyethylene
1937	Poly methyl methacrylate ("Plexiglas")
1937	Polystyrene
1938	Cellulose acetate butyrate
1938	Poly tetra fluoro ethylene ("Teflon")
1939	Polyamide ("Nylon")
1943	Silicone
1948	Acrylonitrile-butadiene-styrene
1956	Acetal
1957	Polypropylene
1958	Polycarbonates

*see Part Two – Microbicide Data

In 1965, Scientific Oil and Chemical Company Inc. introduced the first preservative designed specifically for plastics preservation, 10, 10'-oxybisphenoxarsine (OBPA) [II, 19.1.]. The initial product was a 1% solution in epoxidized soybean oil. The early products were particularly designed for flexible PVC applications. Over the years additional products have been developed with up to 5% OBPA in liquid solutions. In 1976, solid resin concentrates of OBPA were introduced. These products have improved handling convenience. The first isothiazoline, 2-n-octyl-4-isothiazolin-3-one (OIT) [II, 15.4.], was registered for use as an antimicrobial for plastic in 1973.

In recent years, the predominant antimicrobials for plastics include a wide variety of different chemical types. The leading antimicrobials currently being used in plastics include:

- 10, 10'-oxybisphenoxarsine (OBPA)
- 2-n-octyl-4-isothiazolin-3-one (OIT)
- 4, 5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) [II, 15.5.]
- 2, 4, 4'-trichloro-2'-hydroxydiphenyl ether (TCPP) [II, 7.6.1.]

Other antimicrobial agents that find some use in specialty applications in plastics include:

- N-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide (Captan)
- Tri/dibromo salicylanilide (TBS) [II, 10.4.]
- N-Fluorodichloromethylthio phthalamide (Fluorfolpet) [II, 16.2.]
- 3-Iodo-2-propynyl-butyl carbamate (IPBC) [II, 11.1.]
- 2-(4-Thiazolyl) benzimidazole (TBZ) [II, 15.9.]
- Quaternary ammonium compounds [II, 18.1.]
- Phenyl mercuric acetate (PMA) [II, 19.2.]
- Bis-tributyltin oxide (TBTO) [II, 19.5.]
- Tributyltin esters (TBT ester) [II, 19.6.]
- Zinc pyrithione (ZPT) [II, 13.1.3b.]
- N-Butyl-1, 2-benzisothiazolin-3-one (BBIT) [II, 15.7.]
- N-Trichloromethylthio phthalamide (Folpet) [II, 16.1.]
- Silver technologies (glass, zeolite, ceramic, inorganic carriers)

5.13.2 Susceptibility of plastics

In general, plastics are often thought of as being unsusceptible to microbial attack. The degree to which plastics are at risk to microbial attack varies greatly in both severity of susceptibility and in the mode of attack. In some cases, the attack can cause degradation of the polymer chains themselves, an example being some polyurethanes. In other cases, the microbial attack destroys additives, such as plasticizers and lubricants, in the plastic formulation. Loss of these small molecules causes embrittlement, loss of physical properties, discoloration, staining, and odors on the plastic. Cellulosic materials such as wood and other natural fibers increasingly are being used as fillers in plastics. These fillers are another potential food source for microbial attack.

Many types of plastics can benefit from antimicrobial treatment. Flexible PVC is the primary plastic that is treated with antimicrobial. Other polymer systems treated with biocides include polyurethanes, polyolefins, polyamides, polysulfones, and styrenics.

Flexible PVC

Polyvinyl chloride is a versatile material due to the ability of additives to alter its properties. PVC by itself is rigid, brittle, and hard to process. Heat stabilizers, impact modifiers, and lubricants are added to the resin to make rigid products like pipe, siding, and window profiles. The addition of plasticizers allows the material to range from semi-rigid to very soft for such uses as floor covering, wall covering, roofing membranes, swimming pool liners, shower curtains, and refrigerator gaskets.

Plasticizers

The choice of plasticizer has a very large effect on the overall bio-susceptibility of a flexible PVC article. The plasticizer can constitute up to a third of the total material in the formulation and can be the part of the formulation that is most prone to attack. Some plasticizers are highly susceptible to attack, while others show slight to no susceptibility (Table 2).

Phthalate esters are currently the plasticizers of choice for most flexible PVC applications. Di-isodecyl phthalate (DIDP) is the most commonly used plasticizer of this class and in itself is not susceptible to attack. However,

Table 2 Susceptibility of selected plasticizers to fungal attack

Not susceptible	Slightly susceptible	Very susceptible
Di-(2-ethylbutyl)adipate	Di-butoxy ethyl adipate	Di-capryl adipate
Di-(2-ethylhexyl)adipate	Di-iso-octyl adipate	Di-hexyl adipate
Di-decyl adipate	Di-octyl adipate	Mixed n-octyl, n-decyl adipate
Di-nonyl adipate	Di-(2-ethylhexyl) azelate	N-Octyl decyl adipate
Ethyl-o-benzyl benzoate	Di-iso-octyl sebacate	Di-(2-ethylbutyl) azelate
Chlorinated diphenyl	Di-methyl sebacate	Butyl laurate
Chlorinated paraffin	Di-octyl sebacate	Ethylene glycol laurate
Tri-n-butyl citrate		Di-ethylene glycol mono laurate
Tri-ethyl citrate		Glyceryl laurate
Butyl phthalyl butyl glycolate		Sorbitol laurate
Ethyl phthalyl ethyl glycolate		Di-butyl ammonium oleate
Methyl phthalyl ethyl glycolate		Methoxy-ethyl oleate
Di-(2-ethylhexyl) maleate		Tetra hydro furfuryl oleate
Di-butyl maleate		Pentaerythritol ester of caproic acid
Di-ethyl maleate		Barium ricinoleate
Di-methyl phthalate		Butyl acetyl ricinoleate
Di-benzyl phthalate		Butyl ricinoleate
Di-butyl phthalate		Methyl acetyl ricinoleate
Di-decyl phthalate		Methyl ricinoleate
Di-iso-decyl phthalate		Di-benzyl sebacate
Di-nonyl phthalate		Di-butyl sebacate
Di-octyl phthalate		Di-cotyl sebacate
Mixed phthalates		Polyester of sebacic acid
Alkly aryl phosphate		Butoxy-ethyl stearate
Tri-butoxy-ethyl phosphate		Butyl stearate
N-Ethyl, and P-toluene sulfonamides		Epoxidized soybean oil
P-toluene sulfonamide		

the total formulation may be susceptible depending on other additives. Citric, adipic, azelaic, and sebacic esters are used in specialty applications. Epoxidized soybean oil (ESO) is commonly employed at low levels as a plasticizer and secondary stabilizer, and is highly susceptible.

Other additives

Other additives can also greatly affect the susceptibility of a flexible PVC formulation. As is the case of plasticizers, the type of lubricant chosen can affect the microbial susceptibility. The chemical additives that are derived from natural products can provide a rich food source for microorganisms. Microbial attack on these additives can result in a shorter useful life of the product. Some of the most common lubricants employed in plastics are based on fatty acids or fatty acid esters. Paraffins are another common lubricant type. The most frequently used heat stabilizers in flexible PVC formulations are mixed metal salts of fatty acids. Large varieties of other minor additives are sometimes used in flexible PVC, such as antistats, and antiblocks.

Polyurethanes

Polyurethanes are another versatile class of synthetic polymers. They can be made into foam cushion for mattresses, pillows, and carpet padding, or injection molded into shoe soles. Thermoplastic polyurethane can be made into synthetic leather.

Polyurethanes can be classified several different ways

- Thermoplastic or thermoset – cross-linked or not
- Polyester or polyether – based on the linkage in the polyol used
- Aromatic or aliphatic – based on the type of isocyanate used

Each of these variations affects not only their physical properties but also their susceptibility to microbial attack.

The susceptibility of polyester polyurethanes to fungal attack has been well documented (Hamilton et al. 1986). Enzymatic degradation weakens ester and urethane bonds. Subsequent stress, such as stretching or bending, causes visible cracks, which may extend through the thickness of the plastic part.

Polyether based polyurethanes are less susceptible to loss of physical properties due to microbial attack than are the polyester types.

TPE/TPR

Thermoplastic elastomers (TPE) and thermoplastic rubbers (TPR) show a varying level of susceptibility to microbial attack. The oils and other additives present in these materials can provide a food source to the

organisms. In addition, many of these materials have a soft tacky feel and can provide an opportunistic location for nutrients to adhere.

Other plastics

Many plastics are not directly susceptible to microbial attack. This is particularly true of the rigid polymers with few additives. Though some of these plastic formulations do not themselves provide a food source for microbial attack, they are still subject to attack by microbes when exposed to the right combinations of environmental stresses.

Dirt or debris can adhere to the surface of almost any plastic part, or it can accumulate in micro-cracks caused by heat, light, or mechanical stress. Microorganisms can grow on the debris and produce metabolic products that can stain the plastic and give rise to odors.

Applications

A wide variety of plastic products can benefit from the inclusion of a biocide into the formulation. Table 3 lists the most common applications using biocides.

Table 3 Biocides in plastics

Outdoor applications	Indoor applications
Auto Molding Part	Caulk
Awning	Refrigerator Gasket
Tarp	Sheet Flooring
Tent	Carpet Fiber
Pool Liner	Carpet Backing
Ditch and Pond Liner	Wall Covering
Roofing Membrane	Mattress Cover
Window Gasket	Shoe Components
Marine Upholstery	Mops and Mop Buckets
Coated Fabric	Trash Cans
	Air Conditioner part
	Cutting Boards

5.13.3 Cosmetic degradation

Some plastics are subject to microbial attack that can cause polymer staining. The staining can affect the aesthetic value, shorten the service life of the product, or cause premature replacement of the article.

Plastics such as the olefins and rigid PVC may be susceptible to severe staining and defacement as an end result of fungal attack. Others may harbor bacteria, yeast, or molds that produce odors. For that reason frequent cleaning of the surface may be required. If growth conditions are right, fungal growth and associated staining can occur within a few months.

Responsible organism

The microorganisms that are responsible for the staining and biodegradation of plastics include fungi, actinomycetes, bacteria, and algae. Algae that contain chlorophyll can synthesize their own foodstuff from sunlight, air, CO₂, and water. However, algae usually grow on the surface of plastics and do not deteriorate the polymer. Bacteria and fungi cannot manufacture their own food and are saprophytes. They must get their nutrients from dead or living organic matter. These organisms also use their varied enzyme systems to attack susceptible plastic materials. There is another group of organisms that varies in size between bacteria and fungi. They are the actinomycetes that are found in soil. They can cause permanent staining on plastics. Their metabolites can easily diffuse into a plasticized PVC (polyvinyl chloride) article and produce mostly pink and red stains.

Out of all these microorganisms, the fungi are of most concern since they can translocate nutrients through their filamentous structure called hyphae. Masses of these hyphae are known as mycelia. While bacteria need a water phase to survive, fungi can subsist in areas of at least 60% humidity. Temperature can be a factor in determining optimal growth. 70°F to 90°F can be best for some, however we all know that fungi grow well in refrigerators and survive at lower temperatures.

Staining of flexible PVC

A classic example of staining of PVC is due to the actinomycete organism *Streptoverticillium reticulum*, which is often referred to as 'the pink staining organism'. This pinking phenomenon was a particular concern realized by the automotive manufacturers in the 1960's and 70's when vinyl auto roofing or Landau Tops were popular. At that time, *Stv. reticulum* was isolated as the organism responsible for early staining of the roofs that required replacement. This organism, through its metabolic processes produces red pigments that can diffuse through the vinyl via the plasticizer used to make the product soft and flexible. It was reported that prodiginine pigments from *Stv. reticulum* are the probable cause for the pink stains of flexible PVC (Gerber and Stahly, 1975). The presence of the organism in the vinyl system is not necessary to produce the stain. Instead, the discoloration is caused by migration of the stain from a nearby substrate on which the organism is growing. The prodiginine pigments of *Stv. reticulum* are soluble in diisodecyl adipate, one of the plasticizers used commercially by some vinyl manufacturers. Exposure of the plasticizer to red mycelia resulted in extraction of the pigment. Thus, the plasticizer is the probable vehicle through which the pigment is transported from *Stv. reticulum* throughout the vinyl system (Yeager, 1962).

Given the right conditions, the microbial metabolites or dyes are soluble in most plasticizer PVC systems.

For many years, the actinomycetes *Stv. reticulum* had been used as a screening organism in the testing of the susceptibility of plastics. The test adopted by ASTM (American Society for Testing Materials) in recent years utilizing this microorganism is designated as ASTM E-1428-96 (ASTM, 1996).

Other fungi are capable of producing various permanent stains on plastics. They can be blue, black, yellow or purple depending on the causative fungi and growth conditions present on the plastic and the surrounding environment.

Many fungal organisms have been isolated which are associated with stain of flexible PVC. These include the following:

Aureobasidium

Cladosporium

Penicillium

Aspergillus

Curvularia

Alternaria

Helminthosporium

Besides some organisms that can be found growing directly on the plastic part, other components, such as a fabric laminate found in marine upholstery, awnings, tarpaulins etc., or a paper laminate on vinyl wallcovering, can contribute to defacement of the final product. Fungi can grow on these susceptible surfaces and their metabolites will migrate through the plasticized vinyl causing stains on the surface that cannot be removed. Wallcovering adhesive that can support growth of microorganisms can cause staining on the surface of the wall-covering as well as mal odors.

Below are photographs of microbial attack and stains on vinyl surfaces due to actual growth and diffusion of their metabolites.

In addition to these cosmetic damages to plastics due to stains, some may support the growth of many bacteria, yeast and molds that are associated with very unpleasant odors that may make the product undesirable

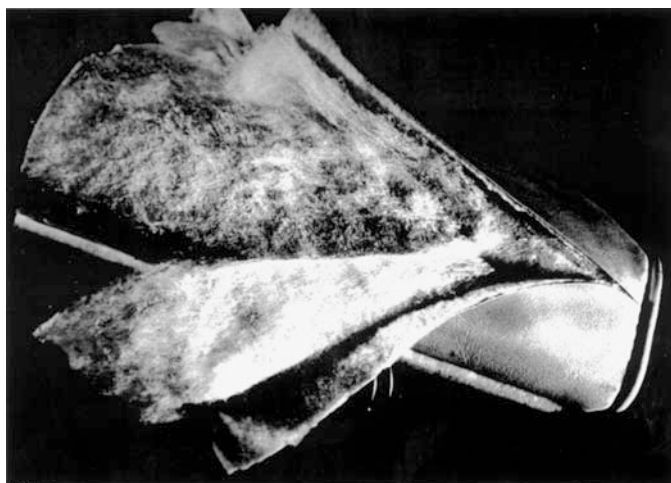


Figure 1 Boat seat with PVC cover non woven fabric and Polyurethane cushion.

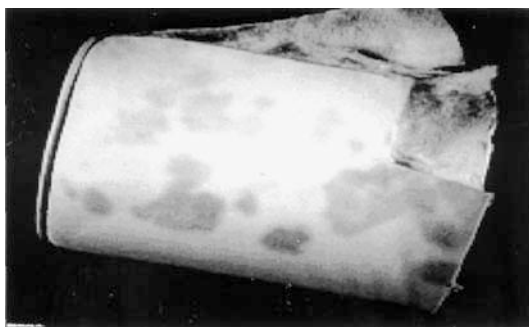


Figure 2 Boat seat with PVC cover non woven fabric and Polyurethane cushion.



Figure 3 Pool liner.

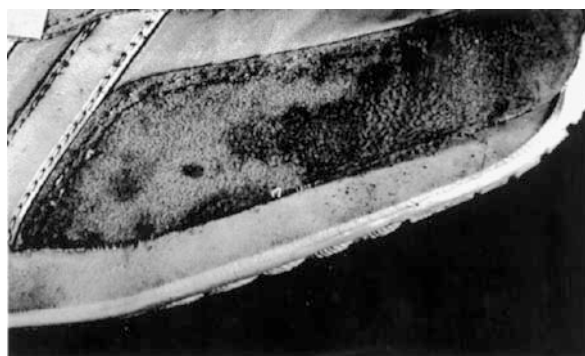


Figure 4 Shoe upper.



Figure 5 Back of wallcovering.

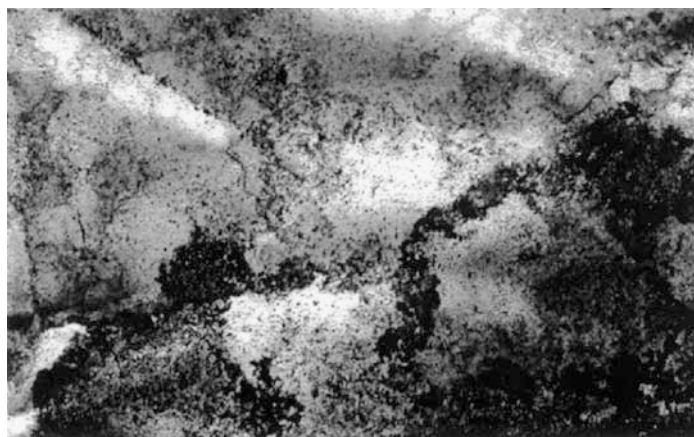


Figure 6 Bottom of pool liner.



Figure 7 Shower curtain.

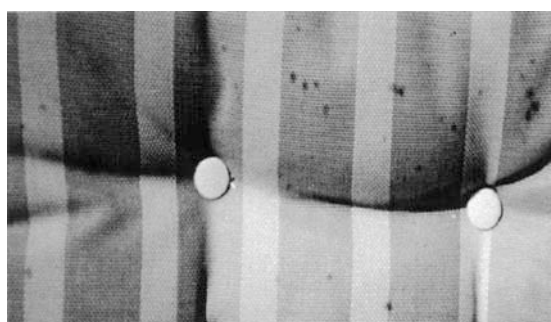


Figure 8 Outdoor furniture.

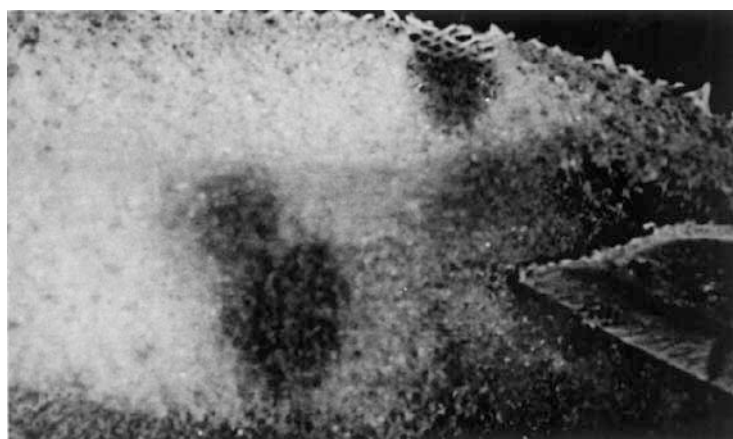


Figure 9 Polyurethane Foam cushion.

to the consumer. These plastics can be a laminate of some sort where the bacteria would thrive in a moist fabric layer. Many vinyl-coated fabrics can wick moisture into the textile section along with indigenous bacterial and fungal organisms. This area is 'protected' from cleaning. The consumer, even though practicing good housekeeping on the vinyl, could experience stains and odors from growth in the fabric layer or scrim.

5.13.4 Structural degradation of plastics

Structural failure of a susceptible plastic can be a significant change in physical, chemical, or electrical properties of the product. Most common structural damages are in polymers such as polyurethanes and flexible PVC. Microbes can attack the polyurethane backbone and break up the plastic molecules creating a failure in the structural integrity of the plastic. Vinyl liners for ponds, roofs, and swimming pools can become brittle and shrink as the organism metabolizes the plasticizers. Plasticizer loss can eventually result in cracking and a subsequent leak in the liner. Below is a photograph demonstrating structural degradation of plastics.

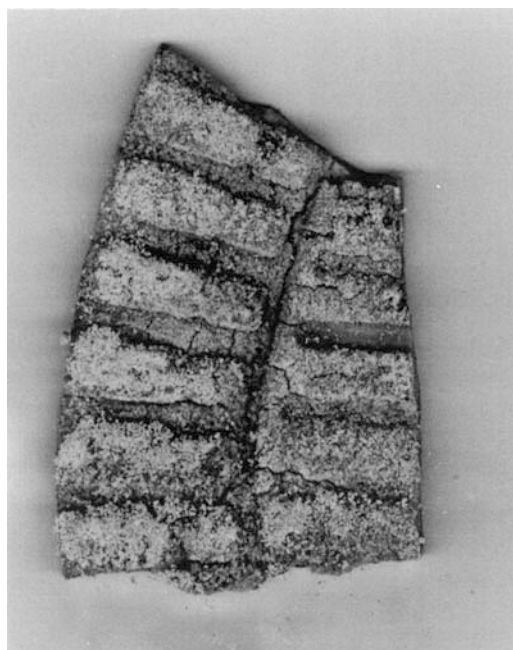


Figure 10 Shoe sole.

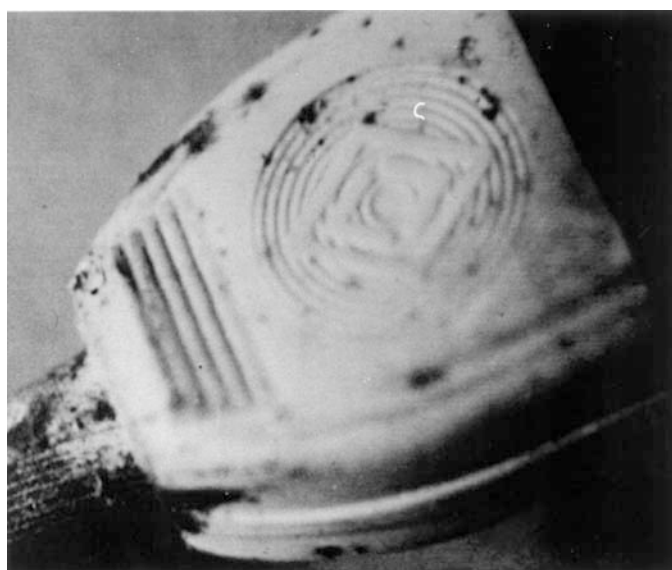


Figure 11 Electric wire.

Action of enzymes on plastics

The deterioration of plastic by microorganisms is due to an extracellular process of enzymatic activity. The enzymes secreted by organisms as part of their life cycle are reactive chemicals that are able to break down large molecules such as plastic and plasticizers into smaller ones since large molecules cannot pass through the organism cell wall. These smaller molecules can be absorbed into the microbes through their cell walls. The cell receives the nutrients it needs via various chemical pathways thus survival and reproduction.

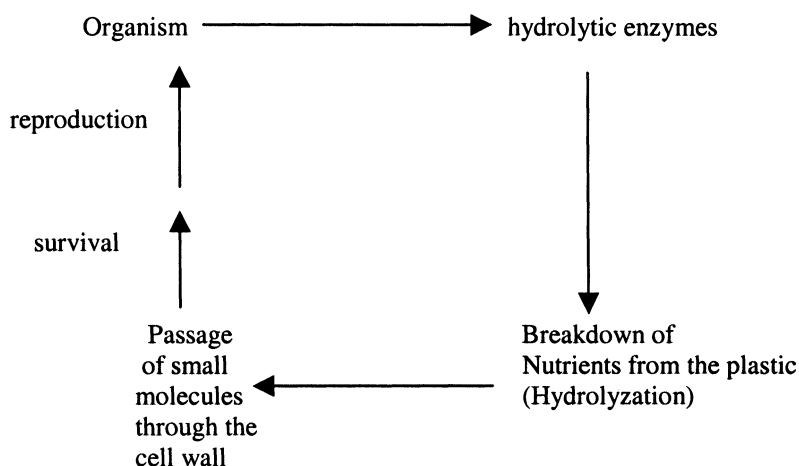


Figure 12 Enzymatic action.

5.13.5 Characteristics of a good biocide for plastics

Due to the numerous factors involved, decision-making is of utmost importance when selecting the right antimicrobial agent for a plastic application and polymer system.

Spectrum of activity against microorganisms

The ideal antimicrobial would have a broad spectrum of activity against many types of microbes. Plastics are used in applications where they are subject to attack from all forms of microorganisms: fungi, bacteria, and algae. There are some applications where this broad spectrum is not required. For example, in roof liners, the predominant attack is by fungi. In cases where bacterial odors are the cause of concern, a bactericide would be acceptable. The selection of the appropriate biocide for an application requires understanding the nature of the biological challenge and the spectrum of activity of the biocide.

Compatibility

The biocide must be both physically and chemically compatible with the polymer formulation. Most biocides work only at the surface of the plastic. For the biocide to be effective, it should be able to distribute within the plastic. The biocide must have sufficient solubility in the polymer matrix so that it can diffuse within the polymer to come to the surface, but not bloom at the surface. Blooming will result in premature loss of biological activity and possibly give rise to unacceptable risk of human and environmental exposure at the surface of the plastic article. Biocides that are chemically incompatible with the additives in the polymer can result in discoloration. In addition, chemical reactions between the biocide and some formulation ingredients can result in deactivation of the biocide.

Dispersibility

Biocides are used at very low levels of active ingredient, typically at about 0.1% or less. In order to be effective the biocide must be evenly distributed over the entire area of the plastic. The use of liquid biocide solutions and solid biocide concentrates help to insure the even dispersion of the active ingredient within the polymer matrix.

Heat stability

Thermoplastic polymers are processed at relatively severe conditions. The process can involve high pressure and sheer with temperatures in the range of 150 C to 250 C. The biocide must be able to survive this process without decomposing or reacting with other formulation ingredients.

Weatherability

Plastic articles are used in applications that expose them to severe exposure conditions. Biocides used to treat plastics for outdoor applications must be able to survive these severe environmental conditions of heat, UV light exposure, rain, and water leaching in order to be effective.

Toxicity

The toxicity profile of the biocide is very important. Biocides that can be leached out of the plastic or bloom to the surface are an exposure risk to humans and the environment. Fish toxicity is also very important in pond and ditch liner applications. The environmental fate of the biocide must also be considered. It is desirable that the biocide is biodegradable and not persistent in the environment after it is done protecting the plastic. Extensive supporting documentation is required on the toxicity and environmental fate of any new product.

Physical form

Antimicrobials for plastics are usually supplied as a biocide concentrate rather than the pure active ingredient because the safe handling of many of the pure antimicrobial agents requires special precautions for which the typical plastic processor is not equipped. The use of concentrates also helps insure that the relatively low level of the additive is accurately dosed and well dispersed into the plastic during processing. Several products are offered as both liquid solutions or polymer pellet concentrates.

Some of the advantages of using liquid biocide solutions include: the ease of metering and transfer from the drum or tank to the blender, quick and thorough dispersion of the biocide into the polymer blend during blending, and compatibility with formulations and processes. Some of the disadvantages of using liquid biocide solutions include the potential for personal exposure through splashing and other contact, the environmental hazard through spills, and the disposal of liquid contaminated containers.

Solid biocide concentrates have several advantages. They are easier to handle safely and clean up of any spilled material is safer and easier than with liquid products. Also, there are fewer problems with the disposal of empty containers. Some disadvantages to solid biocide pellet concentrates can be incompatibility with some customer formulations and processes. It is more difficult to meter small quantities of solid pellets than liquids and solid biocide pellets tend to be more expensive to use.

5.13.6 Evaluation of biocides in plastics

The most common method for evaluation of efficacy of antimicrobials incorporated into plastics is the 'petri dish' test. These tests are qualitative procedures and can be used as a guide to predict the performance of a biocide against various microorganisms. The following methods are used in the evaluation of the susceptibility/resistance of plastics to microorganisms.

Microbiological tests

Laboratory – agar plate or petri dish

- Algae
- Bacteria
- Fungi
- Actinomycetes (pink stain)

Laboratory – simulations

- Humidity cabinet
- Soil burial

Environmental – field

- Outdoor exposure studies

Petri dish methods – bacteria/actinomycetes

Organisms

Staphylococcus aureus

Bacillus sp.

Klebsiella pneumoniae

Escherichia coli

Streptovorticillium reticulum
Salmonella sp.
 others

	Incubation time
<i>Qualitative Methods</i>	
ASTM E-1428-96 (Pink Stain)	14 days
Kirby-Bauer Method (Sherris, 1989)	24 hours
<i>Quantitative Methods</i>	
AATCC Methods 100 + 174 (AATCC, 1993)	24 hours
N.Y. State Mattress Test #63 (NYS, 1992)	24 hours

Note: AATCC (American Association of Textile Chemist And Colorists) oversees textile evaluations however these methods can easily be modified for use in plastics.

Petri dish methods – actinomycetes/bacteria (nutrient media)

Petri dish tests can demonstrate that a plastic part can inhibit or support the growth of particular organisms. Nutrient media is placed in a petri dish and allowed to gel. The test sample is placed on the media previously inoculated with bacteria or actinomycetes. After incubation, the microbiologist measures the 'zone of inhibition' or the area surrounding the plastic where growth of the organism was inhibited. This inhibited zone is due to diffusion of the biocide into the surrounding media.

Bacteria such as *Staphylococcus aureus*, *Klebsiella pneumonia*, as well as Actinomycetes such as *Streptovorticillium* can be used. The test samples are evaluated for microbial stain or growth of cellular colonies in the contact area of the sample.

As mentioned earlier, the pink staining organism *Stv. reticulum* can diffuse its pigments into plastic from the surrounding media or nutrients. This zone repels growth of the organism so that its metabolic processes cannot affect the biocide treated plastic. Some are led to believe that this migration of the biocide leads to rapid depletion of the active ingredient in plastic. In order for a biocide to be effective it must be at the surface of the plastic and be readily available and be replenished.

This 'zone' of biocide diffusion is affected by the following:

- Solubility of the antimicrobial in the plastic
- Temperature
- Vapor pressure
- Physical geometry
- Relative potency
- Solubility of the biocide in the polymer matrix
- Biocide concentration
- Biological challenge

In flexible vinyl, the plasticizer system maintains equilibrium throughout the plastic matrix and facilitates the diffusion of the biocide. As the plasticizer diffuses to the surface, the biocide is always available to inhibit microbial growth.

The zone of inhibition is not a necessary criterion for the plastic to show acceptable performance as long as the plastic surface is free of microbial growth. However, microbial metabolites still may be able to migrate into the plastic part when organisms are in close contact with the plastic part.

If a biocide migrates too slowly, the zone of inhibition may be small or nonexistent in the petri dish test. However, organisms that are attacking the surface can be controlled if the biocide is potent enough (see MIC values of biocides in Part II). As long as a steady migration of biocide continually supplies the surface, resistance is observed. In the field, it would appear that the slower migrating biocides would last longer. However, as the loading of extraneous nutrients mount on the plastic including dirt, debris, organic matter etc., the availability of the biocide at the surface may be too low to effectively control the environmental stress in a short time.

An effective biocide must show low MIC values as well as a minimum diffusion level to the surface of the plastic for maximum field performance.

A good formula in the plastic should provide good mobility for the biocide as well as satisfactory physical properties of the product.

The following photos demonstrate the Kirby-Bauer bacterial resistance test as well as the ASTM E-1428-96 pink staining organism test.

See attached appendix A and B for a synopsis of the test methods.

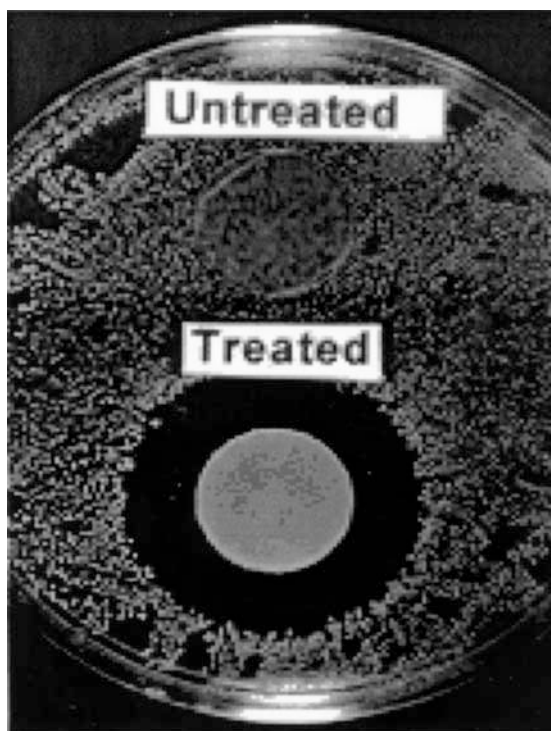


Figure 13 *Staphylococcus aureus*.

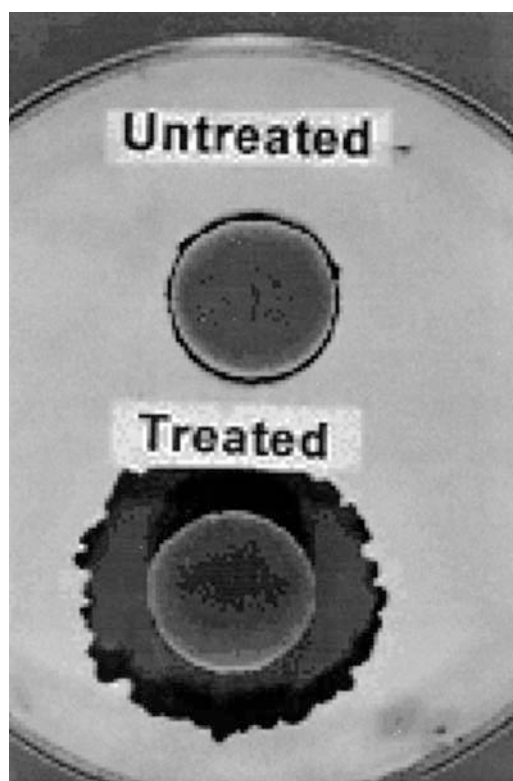


Figure 14 Pink stain – *Stv. reticulum*.

Petri Dish Methods – Fungi (non-nutrient media)

ASTM G-21-96 (ASTM, 1996)

Incubation time: 21–28 Days

Organisms

Aspergillus niger

Penicillium pinophilum
Chaetomium globosum
Aureobasidium pullulans
Gliocladium virens

Other petri dish tests require the use of non-nutrient media and gel. The ASTM G-21-96 procedure is described here. In this test, there are minimal nutrients in the petri dish gel (agar) so growth of fungi on the media is very sparse. The fungal inoculum is forced to search for food, a carbon source of nutrient, directly from the plastic test piece.

The inoculum, consisting of five fungi, is sprayed or pipetted onto the plastic surface. Spraying with a wetting agent is desirable to spread the inoculum evenly without 'beading' on the surface since plastics are hydrophobic. After incubation, the samples are evaluated macroscopically and if needed with the aid of a low powered microscope ($\sim 30\times$) and evaluated for percent surface fungal growth on susceptible materials.

See appendix C for a synopsis of the test method.

See photographs of resistance and susceptibility below:

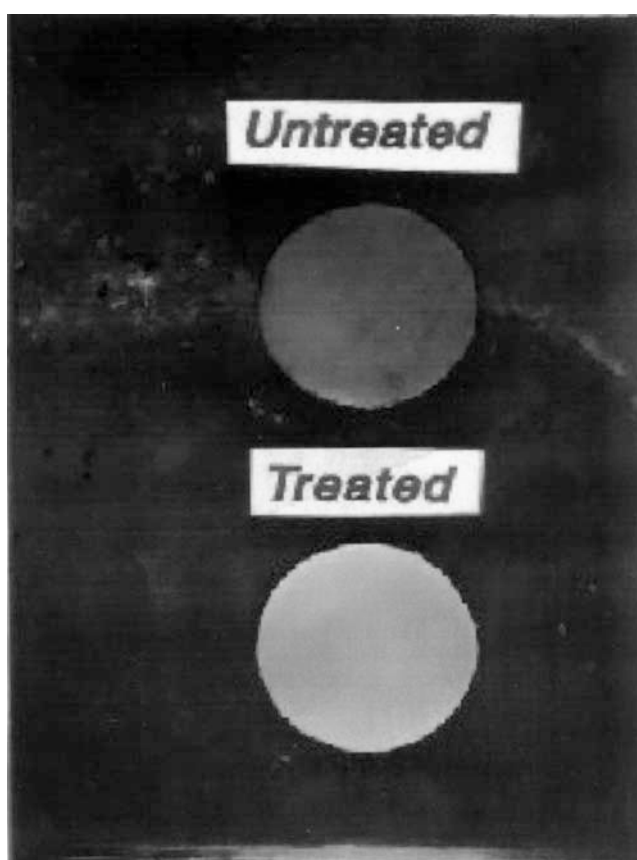


Figure 15 ASTM-G-21-96.

Quantitative test for bacteria

Two widely used tests to evaluate plastics or similar materials (polymeric fibers) measure the percent reduction or percent survival of bacterial cells over a 24-hour period. The tests are:

- NYS (New York State) #63 – Mattress Covering Test – Appendix D
- AATCC (American Association of Textile Chemist and Colorists) Method 174
- Part II – Carpet Materials (adopted from AATCC method 100 – Textiles) – Appendices E and F

The procedures allow the biologist to inoculate the test samples with a known amount of viable bacterial cells of about 10^5 organisms or CFU (cell forming units) in the inoculum.

Recommended test bacteria are:

Staphylococcus aureus
Klebsiella pneumoniae

Other organisms can be used by agreement of the interested parties and appropriate to the end use applications. The samples are inoculated, and then incubated for 24 hours. Following standard microbiological techniques (plate count), the surviving organisms are recovered and the percent reduction is calculated.

The AATCC Method does not specify a pass/fail criterion. Interested parties or industrial trade groups and organizations may set up their own criteria. The NYS #63 has passing criteria of 2% survival for *Staphylococcus aureus* and 17% survival of *Klebsiella pneumoniae*.

Soil burial test – appendix G

Soil burial of plastics is considered one of the most severe laboratory tests run. Not only can microbial stains be observed, severe stiffening of polymers as well as cracking of the material can be seen. Weight loss in flexible PVC can be 10% or higher as well as severe cracking of polyurethane materials.

Since the soil beds are very high in bacterial, actinomycetes, and fungi, a lightweight cotton control can be degraded in a matter of 7 days with a breaking strength loss of up to 90%.

The soil burial test can be found under various designations worldwide. In the USA, one of the methods represented is ASTM D-3083 (ASTM, 1989) typical protocols would range from 30 to 90 days.

See photographs below of polymeric stain and degradation after soil burial.

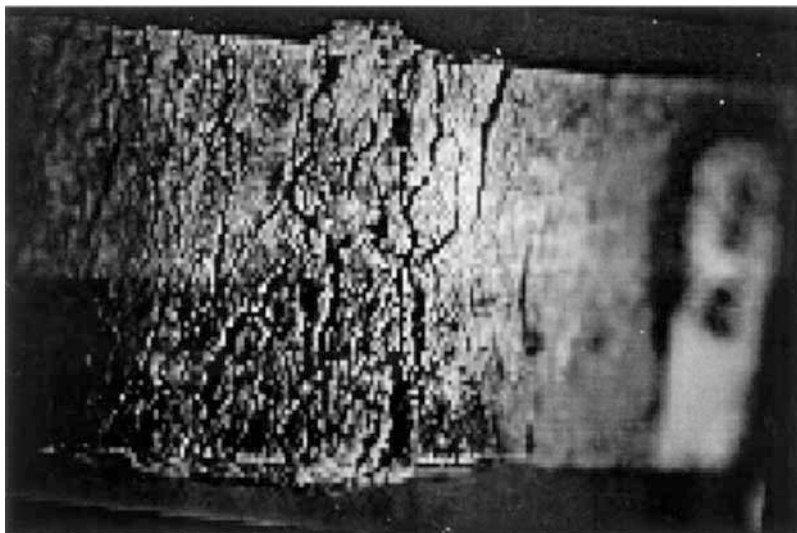


Figure 16 Polyurethane after soil burial (untreated).

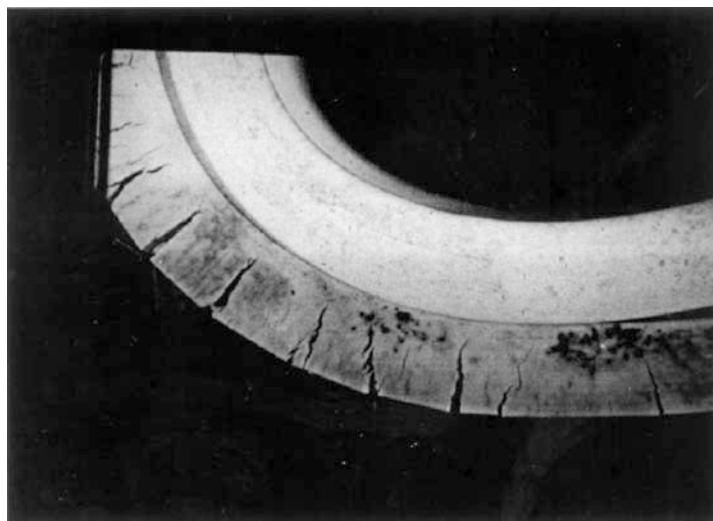


Figure 17 Polyurethane after soil burial (upper half – treated, lower half – untreated).

Algal resistance – appendix H

Vinyl liners, roofing, and the like may be discolored and become unsightly due to attachment of various algal species. In ASTM G-29 (ASTM, 1996), the fresh water alga *Oscillatoria* sp. is used.

This is a good choice of the many algae found worldwide since its dark green color is easy to be observed attaching to polymeric materials. It is a good ‘indicator’ organism to determine how easily test material can support growth.

See photographs below:



Figure 18 ASTM-G-29 test.

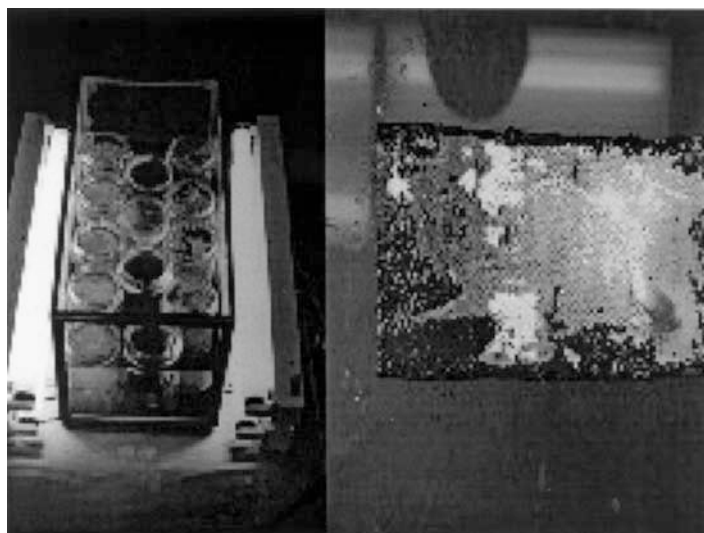


Figure 19 Algal growth on PVC.

Humidity chamber test – appendix I

A chamber with a controlled temperature of ~85–90°F and a relative humidity (RH) of 90–95% is used. This ‘tropical room’ simulates a severe environment that would be expected to be ideal for the cultivation of fungi.

The method MIL-810E Methods 508 (MIL-STD, 1989) was adopted by the military years ago for the evaluation of plastic materials including PVC, polyurethane etc. as well as natural materials for attack and growth of fungi.

After incubation of 28 to 84 days, the materials are evaluated for surface fungal growth. Additional properties can be measured such as hardness, stiffness, breaking strength, and electrical properties.

See photographs below of athletic shoe samples after exposure.

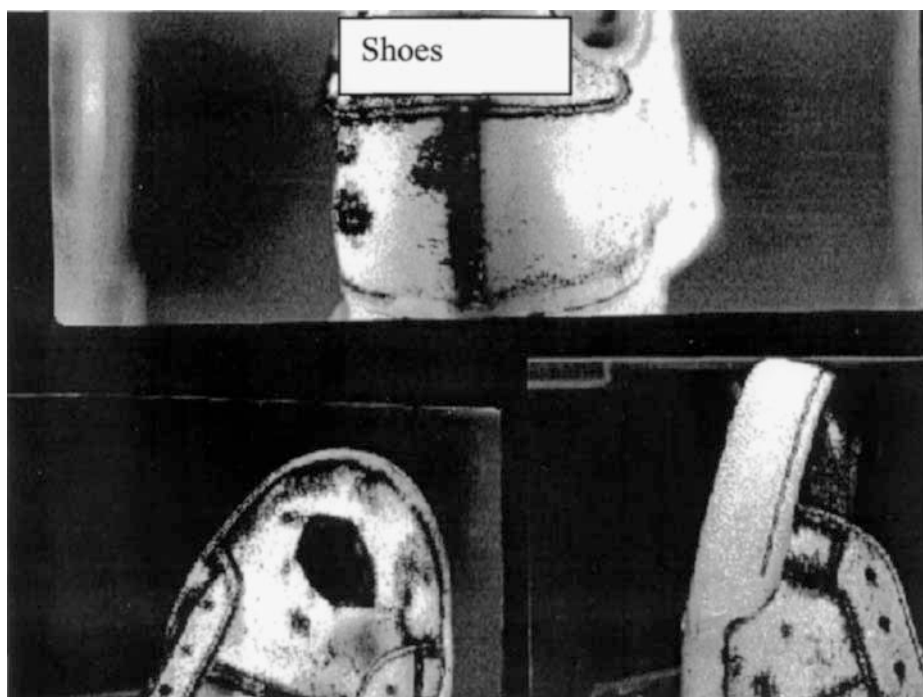


Figure 20 Tennis shoes.

Outdoor exposure

Tropical or sub tropical testing of plastics gives an indication of field performance although variations may result due to a number of factors that can influence test results such as plastic formulations, rain, temperature and other climatic changes.

Exterior testing for certain applications of plastics should be conducted to support microbiological results obtained in initial petri dish screening of biocides. This testing can be summarized as follows:

- Exposures are time consuming. The length of time should be at least a year to obtain a good profile on biocide effectiveness. Biocides should be tested at recommended levels in each formulation along with a similar specimen that is untreated.
- There is not a good control of environmental conditions. The geographical location as well as the season of exposure will affect testing. High humidity vs. low as well as the amount of rainfall is of consideration.
- A trained microbiologist should be used for rating the samples to distinguish between dirt, debris, and mildew as well a discoloration from the environment.

Exposure of plastic film in an environment that represents ideal conditions for growth of mildew will give the best long-term production for products that will be used in various climates around the world.

Plastic materials exposed to climates that are sub-tropical in nature show the following common isolates.

Aureobasidium
Alternaria
Fusarium
Penicillium
Curvularia
Aspergillus
Nigrospora

This is just a partial list of fungi, not all are responsible for severe microbial staining. Likewise not all are able to degrade the polymer surface that would cause premature failure.

Exposure testing is performed in accordance with ASTM G7-97 at a tilt angle of 45° from the horizontal facing south with specimens mounted back. The samples are recovered and evaluated for mildew growth, cracking and delamination.

A synopsis of the ASTM G7 (ASTM, 1997) exposure rating is discussed below with accompanying photographs.

Rating Scale	
None	10
Very slight	9
Slight	8
Moderate	6
Pronounced	4
Severe	2
Very severe	0

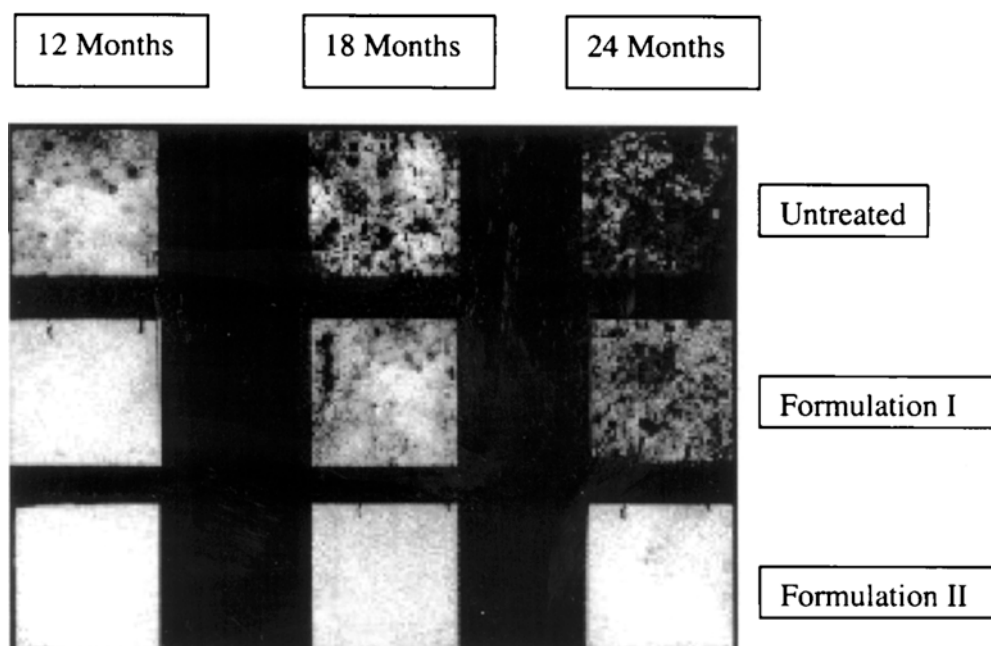


Figure 21 Outdoor exposure - Florida.

Other Applicable Methods

ISO – International Standards Organization

JIS – Japanese Industrial Standards

BS – British Standards

CAN – Canadian Standards

NFX – French Standards

DIN – German Standards

This list by no means covers all existing standards. Many organizations and countries have adopted and revised methods that may be applicable to indigenous organisms, local conditions for the geographic area etc.

Regulatory

Biocides for plastics are regulated in the United States by the Environmental Protection Agency and must be registered as pesticides. Plastic articles that are protected from microbial attack do not have to be registered if they are covered by the treated article exemption of the EPA regulations. The regulations specify the types of claims that can be made for the treated article. On March 6, 2000 the EPA issued Pesticide Registration (PR) Notice 2000-1 “Applicability of the Treated Article Exemption to Antimicrobial Pesticides”. This notice give guidelines for the claims that the Agency is likely to consider acceptable under the treated article exemption. See also Chapter 4.1.

Currently, European legislation on the BPD (Biocide Product Directive) is evaluating all biocide active ingredient chemistries used in plastic and other products. See also Chapter 4.2.

In many other parts of the world, the claims that can be made concerning the benefits of treating plastics with antimicrobials are currently not regulated.

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APPENDIX A

Bacterial Resistance (Qualitative)

Kirby Bauer

The samples are placed on nutrient agar inoculated with:

Staphylococcus aureus ATCC 6538
Klebsiella pneumoniae ATCC 4352

After 24 hours of incubation at 37°C, antibacterial activity is evaluated by measuring (in mm) the size of a clear zone of no growth (Zone of Inhibition) around each sample, and visually determining growth in the contact area.

Bacterial growth is rated by the following scale:

No growth contact area (NGCA)

This is a designation frequently used in bacterial tests. Bacterial organisms are difficult to determine on the sample itself, so the area immediately under the sample is examined for growth. This is usually a passing designation and indicates that no bacterial colonies were found under the sample.

Growth contact area (GCA)

This indicates failure of the sample since colonies of bacteria are detected immediately under the sample in contact with the same.

APPENDIX B

Mildew Resistance

Pink Stain ASTM E-1428-96

The samples are placed on nutrient agar inoculated with the pink staining organism, *Stv. reticulum* ATCC 25607. After 14 days of incubation at 28°C, antifungal activity is evaluated by visually rating the degree of stain.

Surface stain is rated by the following scale.

No Stain	(NS)
Trace of Stain	(TS)
Light Stain	(LS)
Moderate Stain	(MS)
Heavy Stain	(HS)

APPENDIX C

ASTM G-21-96

Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

The samples are placed on (non-nutrient) mineral salts agar and inoculated with a mixed fungal spore suspension of:

<i>Aspergillus niger</i>	ATCC 9642
<i>Penicillium pinophilum</i>	ATCC 9644
<i>Chaetomium globosum</i>	ATCC 6205
<i>Aureobasidium pullulans</i>	ATCC 9348
<i>Gliocladium virens</i>	ATCC 9645

After 21–28 days incubation at 28°C, antifungal activity is evaluated by visually rating the degree of fungal growth on the samples.

Surface fungal growth is rated by the following scale:

No Growth	(NG)
Traces of Growth (less than 10% coverage)	(TG)
Light Growth (10 to 30% coverage)	(LG)
Medium Growth (30 to 60% coverage)	(MG)
Heavy Growth (60% to complete coverage)	(HG)

ASTM Rating

- 0 = No Growth
- 1 = Traces of Growth
- 2 = Light Growth
- 3 = Medium Growth
- 4 = Heavy Growth

APPENDIX D

New York State Spec. #63 (1992)

Mattress Covering Material

The samples are quantitatively evaluated for bacteriostatic activity by placing 0.2 ml of diluted culture of the test bacteria (10^5 organisms) in direct contact with the sample. After 24 hours incubation at 37°C and 100% relative humidity, the samples are diluted with sterile letheen broth and the number of surviving organisms determined by the standard plate count method. The percent survival is calculated by comparison to an untreated control film.

Maximum N.Y. State Spec. Requirements:

- *Staphylococcus aureus* – 2% survival
- *Klebsiella pneumoniae* – 17% survival

APPENDIX E

Quantitative Assessment of Antibacterial Activity on Carpets

AATCC Test Method 174-1991 Part II

The samples are quantitatively evaluated for bacteriostatic activity by placing 0.1 to 0.5 ml of diluted culture of the test bacteria (10^5 organisms) in direct contact with the sample. After 24 hours incubation at 37°C and 100%

relative humidity, the samples are diluted with sterile letheen broth and the number of surviving organisms determined by the standard plate count. The percent reduction is calculated by comparison to the number of organisms recovered at zero contact time.

APPENDIX F

Antibacterial Finishes on Textile Materials: Assessment of AATCC Method 100-1993

The samples are quantitatively evaluated for bacteriostatic activity by placing 1.0 ml of a diluted culture of the test bacteria (10^5 organisms) in direct contact with the sterilized sample. After 24 hours incubation at 37°C and 100% relative humidity, the samples are diluted with sterile letheen broth and the number of surviving organisms determined by the standard plate count. The percent reduction is calculated by comparison to the number of organisms recovered at zero contact time.

APPENDIX G

Soil Burial Procedure

The samples are placed horizontally on a four-inch bed of soil and covered with a one-inch layer of loosely packed soil. After incubation in a chamber maintained at 85°F and a relative humidity of 85 to 95%, the samples are recovered and the microbial stain is determined.

Stain is rated as follows:

No Stain	(NS)
Trace Stain	(TS)
Light Stain	(LS)
Moderate Stain	(MS)
Heavy Stain	(HS)

APPENDIX H

ASTM G-29-96

Standard Practice for Determining Algal Resistance of Plastic Films

The samples are suspended in jars and inoculated with a suspension of the alga: *Oscillatoria sp.* The jars are filled with a diluted salts solution and illuminated by four 20-watt "cool light" fluorescent bulbs for 12 hours each day. At three-day intervals, a fresh inoculum of algae is added to each sample jar. After 14 days at room temperature, the samples are removed and examined for adherent algal growth.

APPENDIX I MIL. STD. 810E Method 508.4

The samples are placed in a humidity chamber.

The samples are then inoculated with a mixed fungal spore suspension of:

<i>Aspergillus niger</i>	ATCC 9642
<i>Aspergillus flavus</i>	ATCC 9643
<i>Aspergillus versicolor</i>	ATCC 11730
<i>Penicillium funiculosum</i>	ATCC 11797
<i>Chaetomium globosum</i>	ATCC 6205

Incubation takes place under a daily cycle of temperature and humidity conditions consisting of 20 hours at a relative humidity of $95 \pm 5\%$ and an air temperature of $30^\circ \pm 1^\circ\text{C}$ ($86^\circ \pm 2^\circ\text{F}$) followed by a four-hour period in which a condition of $95\% (\pm 5\%)$ relative humidity at $25^\circ \pm 1^\circ\text{C}$ ($77^\circ \pm 2^\circ\text{F}$) is maintained for at least two hours. Up to a total of two hours of the four-hour period is used for the transitions of temperature and relative humidity. Temperature and humidity conditions during the transition period are as follows:

Temperature 24° to 31°C (75° to 88°F) and relative humidity of > 90%.

After 28–84 days incubation, the samples are examined by visually rating the degree of surface fungal growth.

No Growth	(NG)
Traces of Growth (less than 10% coverage)	(TG)
Light Growth (10 to 30% coverage)	(LG)
Moderate Growth (30 to 60% coverage)	(MG)
Heavy Growth (60% to complete coverage)	(HG)

5.14 Surface coatings

W. LINDNER

5.14.1 Introduction

Already in the year 15000 b.c. human beings decorated their homes by painting the walls as testified by the discovery of the famous Lascaux Cave in France in 1940. The cave contains an impressive display of prehistoric wall paintings done with mineral pigments mixed with animal fat as a binder. After only 15 years opened to be visited by the public the cave had to be closed again because of microbial growth on the paintings. Algae of the genus *Chlorococcales* were favored to grow after visitors brought into the cave considerable amounts of soil and air pollution.

Today, the design i.e. the form as well as the surface is of utmost importance for the appearance of all man-made goods. Material and products are completely altered in all aspects by applying coatings. Surface coatings protect objects, provide functionality, prolong the life-span and maintain the value of assets. Coatings do not just protect materials but they are the key to beauty, charm, design and emotions, thus increasing the enjoyment of life.

Total coatings industry output worldwide was about 23.6 Million tons in the year 2000 (Ita, 2002) worth about 64 Billion US-Dollars, not including paper and textile coatings.

Europe accounted for 5.6 Million tons, 61% of this figure was consumed for the coating of buildings. Growth rate in the highly industrialized countries are in accordance with change of general domestic product (GDP), in certain Asian countries the growth rate is significantly higher.

A shift to waterborne coatings has rendered the chemical composition of these protective materials more susceptible to microbial attack. Microbial spoilage of paint films and coatings has been estimated to cost 10 billion EURO per annum in Europe alone.

Essentially, the key drivers for new developments in the coatings industry are either to improve performance, or to hold costs down or to comply with the quickly changing legislative environment world-wide. Of course the structural change of the coating and especially the raw material supplier industry to more global businesses leads to an internationalization of the microbiological issues as well. From the marketing point of view microbiocides for coatings are to be considered as typical coating additives affected directly by the trends mentioned above.

Concerns on VOC-emissions are driving the coatings industry from traditional solvent borne systems to high solids, powder coatings or water based paints and to ever lower concentrations of volatile ingredients in these water borne products. The development is differently progressing in the local regions of the world due to different local home construction methods and different traditional painting behavior. EU's goal is to drastically reduce the level of air pollution in the member states of the EU by 2010, thus enhancing the air quality for all the population.

Although scientifically still under discussion it is politicians goal to reduce carbon dioxide emissions to avoid the greenhouse warming effect. Already reality in Europe, in the USA a White House commission recently identified it not only as real, but of growing significance. Everywhere the pressure for conversion of fossil fuels and a need for energy saving will be recognized as increasingly important. This will have a great influence on design, construction and operation of houses and the coating of walls (Pere, 2002). Improved insulation of walls changes the micro-climate on the interior and exterior surfaces of house walls to better growth conditions for fungi and algae, respectively. These developments triggers the desire to protect the surfaces better against microbial defacement.

The start of the development of ready-to-use water based coating materials was triggered by the availability of polymer dispersions as binders. Before that days the water based paints were freshly prepared from industrially produced powdered ingredients and water. Today water based coatings are used for processing paper -, textile -, leather -, metal -, wood and mineral surfaces. As all these coating materials are containing water, a polymer

Table 1 World paints and coatings demand (metric tons) – (Ita, P., 2002)

	1995	2000	2005 (estimated)
North America	5 977 000	7 010 000	8 010 000
Western Europe	4 960 000	5 605 000	6 400 000
Japan	1 956 000	1 851 000	2 060 000
Asia Pacific (excl. Jp)	3 814 000	4 718 000	6 310 000
Rest of the World	4 023 000	4 416 000	5 520 000
World	20 730 000	23 600 000	28 300 000

dispersion, pigments, fillers, rheology modifiers, additives and other ingredients, they more or less are prone to microbial attack in the wet-state.

Water is a pre-requisite for organism growth. As the raw materials used have to be non-persistent water based coating materials or coating films under humid conditions supports microbial growth if no microbistatic or microbicidal chemicals are available.

Some selected chemicals with high microbistatic or microbicidal activity and acceptable detrimental effects versus men and environment are used as preservatives.

Preservatives used in the coatings industry are further classified by their applications: in-can preservatives are used to protect water based materials in production, in-tank and in-can against microbial growth; film preservatives are designed to protect the coating film in service. Masonry biocides are used to remove microorganisms from grown coatings.

Of course the coating materials for different substrates and application are covering a broad spectrum and the susceptibility to microbiological issues is different.

5.14.2 Architectural coatings

The biggest paint market segment for microbicides is the architectural coatings industry. Architectural paints are applied as well in interior as in exterior situations. Construction habits are locally different and that is why the finish materials are coated on very different substrates. In the USA exterior paints are frequently applied on wooden sidings, but brick, stucco, vinyl and metal siding are gaining importance. In Florida and other tropical areas the people are used to paint their roofs in white color. Americans prefer semi- and high gloss paints, which are often containing water reducible alkyd binders. In Germany architectural coatings in general are high PVC mat emulsion paints. Mineral surfaces are found most frequently at houses. Only a few domains are left for solvent borne alkyds or acrylics in Europe. In Scandinavia wood is the preferred substrate and paints are traditionally colored with iron pigments. In South East Asia and other parts of the world freshly erected concrete walls are painted. Here the contractors often start painting the wet and highly alkaline concrete at the bottom while the building is growing the next store. A recent European development for colder climates are the EIFS (exterior insulation and finish system), the most economic version prefers a thick coating (stucco) as the exterior finish layer. As a binder the stucco contains either a polymer dispersion at pH of 8.5 to 9.5 approx., or inorganic silicates driving the pH up to 13. Silicone polymers are used to hydrophobize the finish. In many countries single-family-homes are often repainted after only 1, 2 or 3 years in service by the house owner himself. Big flats are renovated probably after more than 10 years by contractors. Higher living standards trigger a desire to live in a clean environment and the closest environment is the own home. House owners are watching more carefully dirt pick-up or microbial growth on their walls and they want to keep their premises in a light and clean state. Of course the variable climate conditions all over the world provide different ecological niches in-can or on-film that may be exploited by microorganisms. Modern interior coatings are formulated almost solvent free, in exterior coatings typically 2% of a coalescent is necessary. These examples demonstrate the very different requirements on coating materials.

It is clear that the coating formulas for all these requirements have to be different as the preservation systems can not be the same for all applications.

It can be stated that without doubt, the necessity to use antimicrobial compounds is increasing to rationalize production, storage and distribution, to minimize wastes and to keep the functionality and the appeal in service, in order to enlarge renovation intervals and to minimize costs in service of buildings. It was a result of an overall assessment to reduce the VOC-level of paints which would have been impossible without the use of in-can preservatives.

Today low-emission architectural interior coatings are among the materials with highest environmental and toxicological demands on the applied preservatives. That is why only very few molecules can be used as active ingredients in paint preservatives.

This chapter will focus on the most extensive application field, the architectural coatings market; other applications like leather coatings, paper coatings, inks, industrial coatings, automotive paints, textile coatings have some special issues but principles written down about architectural paints can be transferred to related applications as well.

5.14.2.1 Classification of architectural paints

A simple classification of architectural paints can be done by the PVC (Pigment-Volume-Concentration) (Kuropka, 1999). The following examples indicate typical values

Interior Paints	
Highly filled interior paint, wash resistant	85% PVC
Interior mat paint, rub resistant	80%
High quality interior Matt paint	70%
Semi-gloss interior paint	35%
Exterior Paints	
White exterior wall paint	45 to 55%
Elastic exterior wall paint, crack-filling	25 to 45%
Roof coatings	25%
Silicone paint	PVC is not applicable
Dispersion silicate paint	PVC is not applicable
Stucco	Dispersion (styrene acrylate/acrylate/ vina-ethylen-copolymere/VeoVa) Silicone (functionalized Polydimethylsiloxane) Silicate (containing styrene acrylate)
Gloss Paints/Varnishes	
White enamel	20%
Colored enamel	5 to 15%
Wood Coating	
Wood protection paint	30 to 40%
Wood gloss paint	20 to 30%
Wood varnish	5 to 20%
Clear Coat	0%
Primer	
Penetrating primer	0%
Barrier primer	25%
Pigmented stucco primer	50 to 65%

German interior paints are highly filled, exterior paints are in the medium range of PVC and wood coatings at the low end. Semi- and high gloss enamels as well as primers are often formulated based on water reducible alkyd binders.

Today a broad variety of binders is available world wide. For each application alternative systems are possible. Nevertheless there are still local differences in availability and prices for different dispersion types. Of course not only the main monomer is influencing the properties, sometimes minor monomers, e.g. reactive monomers like adhesion promoters are more important also for the compatibility with the applied preservatives.

The higher the binder content in a paint the more important is the binder for the properties of the coating as well as the efficacy of the preservation. As the interactions of the paint ingredients are significant for the properties of the material they are important for the preservation as well. That is why today evaluation of preservative systems is still most often an empirically solved problem, governed by the experience of the investigators and the plant personnel. It is highly recommended to use properly adapted statistical design of experiments for evaluation of preservatives in coating materials (Julian, 2001).

Table 2 Typical paint starter formulas: (Kuropka, 1999)

Ingredient	% in interior paint	% in exterior paint	% in white enamel	Function
Water	30	5 to 12	5	Diluent
Cellulose ether (HEC, MHEC, CMC)	0.3 to 0.6	0.15 to 0.25	0.1 to 0.25	Thickener
Polyphosphate	0.05 to 0.15	0.05 to 0.1	0	Co-dispersant
Polyacrylate	0.2 to 0.5	0.2 to 0.5	0.5 to 1	Dispersant
ammonia (25%) or alkali solution (10%)	0.2 to 0.5	0.2 to 0.5	0.2 to 1.5	pH-regulator
Titanium oxide	5 to 15 (Anastas/Rutil)	5 to 15 (Rutil)	15 to 25 (Rutil)	White pigment
Carbonate + Silicates	40 to 60	20 to 40	0	Extender
Polymer dispersion (50%)	8 to 18	20 to 40	50 to 70	Binder
Defoamer	0.3	0.3	0.3	Additive
Film forming agent/solvent	0 to 2	1 to 3	0 to 10	Coalescent
Acrylate/PU	0 to 0.5	0 to 0.5	1 to 4	Associate thickener
In-can preservative	0.25	0.2	0.25	In-can preservative
Film-preservative	0	0.1 to 1.5	0	Fungicide-Algicide

In cheap types of ceiling paint, carboxymethyl cellulose (CMC) is used as a thickener and binder in concentration up to 3 wt-%. As the pigments are a significant cost factor in the paint production a tendency can be observed to use as low titanium dioxide as possible. Of course these factors are influencing tremendously the preservation. As a rule of thumb it can be stated that the lower the raw material costs and the poorer the quality of raw materials the more efforts have to be put on a safe preservation system, the easier a carbohydrate based thickener is biodegradable, the more efforts have to be put on the microbiological stabilization.

A special preservation problem solvent free tinting pastes are to be recognized, which are often handled in special tinting machines at retailers and which are in risk of fungal spoilage. These materials are not easy to preserve and each color has to be treated separately. Especially the biocidal actives are adsorbed by the large surface of carbon black and might be destroyed by a catalytic effect.

A typical architectural paint production is a two-step batch process, the grind and the let-down. In a pre-step the thickener is often dispersed and swollen by water up-take. The grind stage is done in a dissolver. Water and dispersants are mixed with the pigments and extenders and at high shear rate the solids are dispersed until free of aggregates. This step takes typically only a few minutes to hours. The letdown stage is done in a mixing tank, the binder and the additives are added at low shear rate. Preservatives are handled typically like other small volume additives.

Paint plants are characterised by storage tanks for liquid raw materials and products connected to the production units by pipes, they have silos for powdered raw material and filling units for the final packaging into buckets and pails.

5.14.2.2 *Environmental regulation*

Different environmental regulations for antimicrobials cause different use patterns for biocides in different countries of the world.

While the USA fell behind in the development of modern preservatives in the 1980s due to FIFRA-Regulations and the cost-intensive approval of biocides by EPA, in Europe a culture of using problem-oriented formulated biocides with optimized spectrum of activity developed. In general Asia was following the European use pattern.

It can be predicted that the European Biocidal Product Directive will slow down the development of formulated and combined preservatives as the hurdles for registration of preservatives will be 100000 Euro approximately per biocidal product.

The consequences of the BPD will be a loss of flexibility to adapt preservatives to the coating material or to the intended use. Either the paint producer will handle himself the concentrated unformulated biocidal actives each separately or he has to cover his microbiological problem with one of the few standard formulations in the market, probably at higher concentrations as it would be necessary with a well adapted one. Further regulations like VOC-limitations from construction materials, indoor-air monitoring, chemical law triggering classification and labeling of preparations, or industrial or consumer labels like the "White Swan" in Scandinavia or the RAL UZ-sign 102 ("Blue Angel" Label in Germany) to name only a few are limiting already today the possible active ingredients and the concentration of preservatives.

In contradiction to the effects of the regulatory measurements these limitations require even more than in the past the use of combined formulated preservatives.

5.14.2.3 *Microbicides in paints – historical background*

Traditionally, no special biocides were applied in the early times of industrial coating material production, because typical paints were based either on alkyd binders dissolved in organic solvents or on inorganic powdered materials which were used by the painter to prepare the paint at site by adding the appropriate quantity of water.

The old alkyd paints contained heavy metal salts (lead) acting as curing catalysts which rendered the paint film microbicidal. It should not be under-emphasized that customer perception changed over the years as well and appearance of a paint film to be acceptable is different today from ancient times.

Even the first emulsion paints emerging in the 1950s were not very susceptible to bacterial growth because the polyvinyl acetate binders used contained sufficient amounts of the free monomer vinylacetate and the hydrolysis product acetaldehyde (II, 2.2.)* to prevent growth. In the 1950s phenylmercury compounds (II, 19.2.-3.) were used in low concentrations of 0.05 to 0.15% as a microbicide for the wet and the dry state, respectively. Alternatively, formaldehyde was very efficient to keep paints protected against bacterial growth, but in practice

*see Part Two – Microbicide Data

the high reactivity and the high volatility of formalin (II, 2.1c.) triggered a switch to the more stable formaldehyde-releasing agents (II, 3.) which are facing a renaissance in recent times in the German paint market. Chloroacetamide (II, 17.1.) was recognized as a very reliable in-can-preserved for emulsion paints in the early fifties and has still its merits due to its temperature and pH-stability. It was proven to be very efficient to keep the spores adhering on the extenders at bay. Other microbicidal molecules, like for example phenolics, found less broad application due to discoloration or unfavorable phase distribution (availability in the water phase of the multiphase emulsion paints).

Two major trends were seen in the 1980s in the Americas and Europe: The emerging of the isothiazolinones, especially 5-Chloro-2-methylisothiazolin-3-one/2-Methylisothiazolin-3-one 3:1 mixtures (CIT/MIT 3:1) (II, 15.3.) as in-can preservatives provided a new standard in paints as well as in other water based products. Due to the high performance at rather low cost only very few other molecules could keep their position in systems, the isothiazolinones were not stable enough in. Using CIT/MIT allowed paint makers to produce heavy metal- and formaldehyde-free products.

Outphasing of phenyl-mercurials opened in-routes for non-metallic film-preservatives into the coatings market. The next generation of microbicides had some advantages like low toxicity and better environmental fade as well as higher selectivity to the target organisms. However, these molecules, mostly spin-offs from plant protection developments, lack the all round performance of the metal-organics, which were used close to a "one shot silver bullet" to prevent all microbial growth in production, in-can and on the finished paint film. Today, separate and distinct molecules have to be added cogently to the paints, which are providing selective bactericidal, fungicidal and algicidal function either in the wet state or on the paint film. Actually, no single active ingredient has a broad enough spectrum of activity and appropriate physical features to be used safely and economically as a sole preservative.

Blending of the active molecules is today's main tool to comply with the demand for safe, cost effective protection of a coating from the cradle to the grave against biodeterioration. Formulated preservatives basing on more than one active ingredient have a deep market penetration in Europe, Asia, Latin America and other parts of the world, while the USA-market was dominated by single-molecule microbicides. This was due to the stringent Environmental Protection Agency (EPA) registration requirements. Only recently, starting in the late 1990s, some of the most successful "world-formulations" were made available in the USA. This development to world preservatives seems to be triggered by increasing registration demands in other countries of the world. The European Biocidal Products Directive (BPD) will be a higher registration hurdle for biocidal products than today's USA EPA – registration. It can be expected that the present European practice to develop microbicide formulations adapted to customer requirements will move more to the USA practice to have only very few different preservatives available.

After the European BPD will be in full force probably only some standard preservative formulations will be available world-wide because high registration cost will not allow to formulate optimized niche products. In addition consolidation of the biocide industry is an inevitable result of the consolidation in the coatings industry and the development of more legal hurdles in the industrialized regions of the world. As registration requirements are developing towards those already in force for plant protection agents, the much smaller market for paint microbicides cannot bear the costs for new molecule development. It can be foreseen with high probability, that no new molecules will be developed for the coating biocide market in the future. The new EU-Biocidal Products Directive will trigger costs of about US-\$ 10 Million for a molecule before the first ton could be sold. The only chance for something new might be a spin-off from other applications. On the other hand the demand for preservatives will grow quicker than the paint market due to the demand for higher quality and longer service time of the architectural coatings. However, some emphasizes were put already on the development of "preservative-free" paints in Europe, but it has to be pointed out that the use of biodegradable raw materials trigger the necessity to poison the materials for microbes, at least by increasing the pH-value far beyond 11, which, however, is not "skin- or mucous membrane-friendly". Using microbicides in coatings provides most often the least health or environmental impact to keep a coating material in a good condition.

Producers of exterior coatings are frequently lamenting the higher susceptibility of coatings against microbial defacement today. There are real reasons for this assumption; since the lack of any heavy metals from driers, pigments or impurities, all ingredients are not persistent in the environment and functional natural based additives like cellulose derivatives were introduced. A very important change was done with the heat insulation techniques applied, turning the surface micro-climate to much more favorable conditions for algal (and fungal) growth. Another reason for more complaints is the growing consumer expectation which does not accept dark spots and color change at rather new house walls.

Indications are found that the change of air pollution as consequence of more exhaust air cleaning technology installation and the cease of burning coal in power plants decreased significantly the average sulfurdioxide air concentration in Middle Europe or Great Britain and shifted the climate more to be in favor for algae and mold to grow on surfaces.

5.14.3 Today's microbicides in the paint industry

Four different types of biocide applications are found in the architectural paint and coatings industry today which are fundamentally different from each other and will be discussed separately:

- * In-can preservation (wet state antimicrobial protection)
- * Plant hygiene (Production and storage hygiene measurements)
- * Film preservation (dry film protection)
- * Dry Film sanitization (fungicidal/algicidal washes)

5.14.3.1 In-can preservation

Bacteria are the main causative organisms for the spoilage of water based paints, although occasionally yeast and fungi cause biodeterioration in the wet state. (Bravery 1988; www.ibrg.org).

Microbial spoilage and growth of viable cells can have any or all of the following consequences for a paint well known from everybody's experience with food:

- malodor, typically for spoilt paint
- gassing
- discoloration by pigmented microbes or by colored metabolites,
- viscosity loss as cellulose based thickeners are enzymatically degraded by bacteria or mold
- ropiness by slime forming Enterobacteria or fungi
- phase separation

Prevention is the only chance; reclaiming from microbiologically spoilt paint is a difficult, labor intensive, often impossible task. For the production units it is an even much more serious threat to spoil the production and bulk storage units by contaminated coating materials. Interruption of production for sanitization is never welcome in the plant and implies high costs.

Customer claims on spoilt paint can cause serious damage to the reputation of a brand. Especially professional users are dependent on high quality coating materials to keep their labor costs down. If recognized, spoilt material has to be re-collected from retailers in a very cumbersome procedure.

The main causative organisms for water-based coating materials are bacteria, although yeast and filamentous fungi can also cause biodeterioration. Fungal growth on liquid paints is usually a surface phenomenon (Bravery, A.F., 1988), as fungi need oxygen to grow. Filamentous fungi are frequently observed in closed containers on the paint surface in a small layer of condensed water at higher water activity than the bulk paint itself. Condensed water under the lid in production dissolvers, bulk storage tanks or tins supports fungal growth as well.

The main sources of microbial contamination are the water supply and the raw materials used in production, or most often spots of grown paint or raw materials sitting somewhere in an unproper cleaned and monitored production equipment. A lot of work was undertaken over several years by researchers within the International Biodeterioration Research Group (IBRG, www.ibrg.org) to identify microorganisms isolated from paint. From work of this group and further publications the most troublesome microorganisms in architectural paints can be compiled:

Actinomycetes;

Bacteria:

- Alcaligenes species
- Achromobacter species
- Bacillus species
- Escherichia coli
- Micrococcus luteus
- Proteus vulgaris
- Pseudomonas species
- Serratia marcescens

Yeast:

- Candida albicans
- Rhodotorula rubra
- Saccharomyces cerevisiae

Fungi:

- Aspergillus species
- Fusarium species

Geotrichum species
 Penicillium species

From the list it can be concluded that there are no special bacteria responsible for paint spoilage, but almost the whole spectrum of microbes thriving in the environment can be found in these artificial media as long as the conditions are favorable for them.

It has to be emphasized that the Koch's rules are applying to paints as well. A significant microorganism for paints has to be isolated from a paint and it has to be proven that it is capable to grow in the paint and causing the spoilage of the material. An underestimated factor is the succession of microbes in a system. The first invaders overcoming the inhibiting action of microbicides are changing the system by their metabolism. The water activity is changing in cases of phase separation, oxygen is used up giving anaerobically growing bacteria a chance to thrive, the pH is shifting and degradation products are often excellent nutrients for other microbes dormant in the material in very low counts, not detectable by standard techniques. Frequently, at the time of investigation, the first invaders are already died out as a result of the changes in their environment and the causative microorganism is not detected.

From heavily contaminated paint most often several different bacteria strains and other micro-organisms are isolated in parallel. A simple identification of the microbes found in a spoilt paint must not necessarily reveal the causative first invader. The best but most elaborate tool is to trace back the contamination in the production chain, down to the raw materials and intermediates.

Inorganic pigments contain only very low counts of bacteria. Extenders, especially of natural origin, are commonly contaminated with high counts of spores of the family Bacillus, Sarcina and by actinomycetes. Spores are very problematical in a paint, because they are not easily killed by preservatives and have to be inhibited to thrive out over the whole storage period of a paint. This causes a lot of trouble as the concentrations of rather unstable electrophiles, like 5-chloromethylisothiazolin-3-one or bronopol, decrease during the storage period, providing the lower a level of inhibition the longer the storage period. In paints having a high load of spores from the extenders chloroacetamide might be almost irreplaceable for save control.

From the above, it is obvious that a paint preservative must have an unspecific activity against a broad spectrum of environmental important micro-organisms.

Malodor and gassing in production and in the can are most often caused by facultative anaerobically growing bacteria of the family Enterobacteriaceae. Gram-negative bacteria of the genus Escherichia, Enterobacter, Klebsiella, Proteus can form hydrogen sulfide with a pungent odor in a paint. Carbonic acids and carbo-hydrates (degradation products from thickeners) are reduced under anaerobic conditions to carbon dioxide and hydrogen; pungent smelling low carbonic acids are formed giving the "cheese-like" smell of spoilt paints.

Pseudomonads convert proteins to bad-smelling amines.

Under anaerobic conditions sulphate reducing bacteria are able to form hydrogen sulfide.

Anaerobic conditions are not only found in closed containers but due to the high viscosity and slow gas exchange rates, they might be formed locally within the bulk of the paint. Thriving facultative aerobic bacteria are using-up locally the available oxygen and their enzymatic system shifts to anaerobic metabolism.

The importance of biofilms in this process can not be overestimated. There are very good reasons to believe that bacteria are not free floating in a paint, but most of them are living in biofilms or are adhered to surfaces of the ingredients and the equipment. Biofilms in production equipment like tank surfaces, water pipes, on sealants, on filters are built easily and quickly within hours. They can not be removed by simple means but can cause consecutive and unpredictable introduction of locally high microbial counts and biological material in the paint. See also chapter 5.1.

Microbial discoloration of paints is most often due to reactive metabolism products. A black layer is observed after reaction of the a.m. hydrogen sulfide with traces heavy metals. Yellow to brownish discolorations are frequently observed if thickeners are degraded. Colored pigments are produced by several micro-organisms, yellow pigments might be due to Flavobacteria species, red pigments are produced by the yeast Rhodotorula rubescens.

Change of the rheological properties and phase separation is a severe feature of microbial growth in the paint because the properties, the paint was designed for, are lost. Especially enzymatic degradation of thickeners and stabilizers are the reason for loss of viscosity, serum formation and disintegration. Recovery of thickener-degraded paint has to take into account, that killing the bacteria present is not sufficient if extracellular enzymes are released into the paint continuing the degradation of eventually added thickener. An additional enzyme blocker has to be applied as well.

Slime-forming bacteria might cause clogging of pipes in production or inhomogeneity of the paint. Most often scraps of biofilms built up in the plant are responsible for these inhomogeneities in the products.

Bacteria mentioned above might be responsible for pH-shifts by secreting acetic acid, lactic acid, formic acid, succinic acid and other. Pseudomonads are using up lower acids and the pH can drift to higher values. Of course these pH-shifts can be the reason for the change of rheological properties as well.

It is discussed that painting with microbially contaminated paint might cause a health risk for the painter. Direct infection with pathogenic bacteria is of only very low probability, but spores and toxins built by micro-organisms might cause allergic reaction in sensitized persons especially by inhalation.

The necessity to prevent microbial growth in water based paint is no point of discussion. All water based paints are susceptible to bacterial growth unless the pH-value is higher than 12, which could be achieved in silicate based coating materials. Even these high pH-paints can be spoilt after the first use and reclose of the container, because carbon dioxide from the air shifts superficially the system to lower pH-values. Open cans should be used up immediately. The most economic way to cover the risk of microbial contamination is to use an efficient in-can preservative in combination with a proper microbiological control of raw materials, intermediates, packages and production processes. The preservation of a coating material has to be assisted by the choice of insensitive raw material chemicals as far as possible. High concentration of easily degradable cellulose ethers should be avoided. The lower the concentration of low molecular solvent, plastisizer, dispersant and other additive molecules the more important are special additives, like surface active ingredients or biodispersants, to assist the action of microbicides and to transport the microbicidal agent to the bacterial cells in a multiphase system like a paint.

5.14.3.2 *In-can preservatives*

Desired properties of in-can preservatives are defined by:

- Broad spectrum of activity, the product should be active against bacteria, yeast, fungi known to be spoilage organisms in paint and the respective raw materials
- Cost effectiveness
- Stable under pH conditions of the paint (8 to 9.5, or even up to 12 in silicate systems)
- Fast enough speed of kill
- No discoloration
- No viscosity influence
- Low odor
- High water solubility
- Low partition coefficient (pK_{ow}) between organic materials and water
- Low toxicity
- Easy handling in the plant
- A reasonable ecotoxicological profile
- Regulatory compliance

However, there is no chemical entity available meeting all these criteria for all scenarios. In the world of ever more stringent regulatory requirements new molecule development for industrial preservatives is becoming very unlikely. The development costs are by far not justifiable by the markets. The development will be just the other way round, that fewer molecules will be available in the future. There is a threat that work-horses for paint preservation will be available no longer for certain applications, like e.g. interior coatings, because of risk concerns. If for example, the use of 5-chloro-2-methylisothiazolin-3-one in interior paints is becoming even more suspect to cause sensitization, the spectrum of active ingredients used in the industry will shift to higher cost active ingredients already in the market, but not to new molecules. Due to different regulatory requirements in the USA the paint industry is used to single-active-ingredient preservatives, while in the rest of the world combination of active ingredients is the best way to improve breadth and depth of activity. The strategy of combined active ingredients is also the method of choice to cover gaps opened by unstable molecules like the isothiazolinones against secondary spoilage after longer storage periods. In general in-can preservatives for the coating industry are formulated products handled in the plant like other additives.

Active substances. Target specificity is a non-desired property of a technical microbicidal active molecule. The applied molecules are either classified as membrane-active substances or as reactive electrophiles. Some molecules can not be clearly assigned to one of the two categories while showing both modes of actions. See chapter 2.

Membrane-active molecules act via non-specific adsorption on the microbial cell membrane and disturbance of the embedded proteins by influencing the penetration of ions and organic molecules and by inhibition of the ATP-synthesis.

Electrophiles react with nucleophilic functional groups in the cells in particular with enzymes and other proteins. Typical electrophiles are formaldehyde, its releasers and molecules with activated halogen atoms, e.g.

- chloroacetamide [II, 17.1.]
- 5-chloro-methylisothiazolin-3-one (CIT) [II, 15.2.]
- 2-methylisothiazolin-3-one (MIT) [II, 15.1.]

- 1,2-benzisothiazolin-3-one (BIT) [II, 15.6.]
- 2-brom-2-nitropropan-1,3-diol (Bronopol) [II, 17.14.]
- 1,2-dibromo-2,4-dicyanobutane (DBDCB) [II, 17.18.]

Metal salts like

- silver chloride
- copper nitrate

can be classified as electrophile as well and are used sometimes to block enzymes, like cellulases causing rheological changes in paints.

Membrane-active substances used in paint in-can preservatives are e.g.

- glycols, like e.g. Phenoxyethanol [II, 1.7.]
- alcohols, like e.g. Benzylalcohol [II, 1.4.]
- quaternary ammonium salts, like e.g. benzalkonium chloride [II, 18.1.]
- phenol derivatives, like e.g. ortho-phenyl-phenol [II, 7.4.1.] and para-chloro-meta-cresol [II, 7.3.1.]

The modes of action are indications for possible deactivation paths in complex matrices like paints. Membrane active molecules could be adsorbed by interphase surfaces or by surfactants. Electrophiles react typically with ubiquitous molecules like hydroxide ions, ammonia, amines, sulfides and the like. As the first approach the reactivity is related to the antimicrobial activity and that means the higher the activity the more care must be taken to the compatibility with the system.

While emerging in the market in the early 1980s the 3 to 1 mixture of 5-chloro-2-methylisothiazolin-3-one/2-methylisothiazolin-3-one (CIT/MIT) gained quickly the position of being a standard preservative in the coatings industry. Today the majority of water-based paints are containing CIT/MIT at least as part of the preservation system or as residue from the raw materials, respectively.

Formaldehyde releasers (II, 3.) are the second large group of active ingredients used either alone or in combination with CIT/MIT. Properly optimized combination products of CIT/MIT and a formaldehyde releaser provides in a lot of scenarios the best economics for a safe paint in-can preservative.

Discussing indoor sources of chemical emissions, paint is an important factor emitting volatile organic compounds into the interior environment even for longer periods of time.

Room ventilation is the most effective measure to decrease air concentration of formaldehyde emitted from paints quickly. Formaldehyde emissions from an interior paint are no issue for the users of the painted rooms. Of higher concern are today the semi-volatile ingredients with a boiling point above 250°C like e.g. the plastisizers but also microbicidal active molecules, though emitted in low concentration but over much longer period of time. Studies of the emission behavior are performed in small chambers according to ASTM 5116-97. Realistic estimations can be obtained from air sampling of real coating situations indoor.

pH-stability. The modern active ingredients dispose of a built-in instability to fulfill ecotoxicological demands, i.e. after the in-can preservative has done its job, the activity should disappear. This happens normally by abiotic and biological degradation in the environment. A very important abiotic degradation path is the hydrolysis. Of course, in a water based material, microbicide hydrolysis starts already after incorporation. By choosing the right biocide for the material to be preserved and for the storage time it should be preserved, the pH-instability of a biocide turns into an upside. If a pH-labile preservative is used, the preservative should be added only in the production step the pH is already below the hydrolysis limits. For example, if in paint production thickener pre-batches are prepared at pH-values beyond 10, CIT/MIT-based preservatives should never be added to this production step.

Temperature stability. What was mentioned under pH-stability holds true for temperature stability, too. In addition to the temperature conditions in the production process of the material, the storage temperature of the preservative itself should be regarded. Some of the CIT/MIT-based preservatives should be stored in any case below 40°C to prevent degradation even before the preservative reaches the material it has to protect. In hot climate countries it might be advisable to consider a more stable alternative active ingredient. For other actives too some limits in temperature stability exists, for example the formaldehyde disappears the quicker the higher the temperature. pH-value and temperature are no issue while using BIT or chloroacetamide based preservatives as these molecules are much more thermo- and pH-stable.

Low toxicity. The well known phrase of Paracelsius is valid until today: “all materials are toxic, but the toxicity is a function of the concentration they are ingested with”. The handling of preservatives is the same as with other paint additives, they should be handled in the plants with the same directions and precautions as other chemicals. Special handling advices can be found on the label on the drum and in the MSDS. The low use concentrations of the preservatives normally have no impact on the labelling requirements of the goods preserved.

Low ecotoxicity. Because preservatives are used with the intention to kill microorganisms, the products are dangerous for microbes in the environment. As mentioned for the human toxicity the microbiocides lose their activity when diluted below the no-effect-level. The most important outlet into the environment is the waste water treatment plant (WTP). It is important to keep in mind that the elimination of xenobiotics in the WTP is strongly depending on the adaptation of the bacteria. Bacteria adapt their enzyme battery if faced to sub-lethal doses of a microbiocide.

Compatibility. The preservative must be compatible in all aspects with a wide range of the chemicals used in paint production and should influence neither the color, the odor, the viscosity nor the rheology of the matrix. An important aspect is the reaction of the microbiocide with constituents in the paint to be preserved. Especially for the CIT/MIT-based preservatives even trace impurities in other raw materials are able to destroy completely the activity of the preservative. On the other hand ingredients in a coating formula can assist to keep the level of free formaldehyde splitting off from a preservative containing formaldehyde reaction products below limits.

In polyphasic systems like paints, the active ingredient can migrate into organic phases or can be adsorbed on interphase-surfaces and can be de-activated. This de-activation mode is typical for membrane-active microbiocides like the quaternary ammonium compounds.

In general a preservative should be added to the product as early as possible in the manufacture. Depending on incompatibilities in early stages of the production process, e.g. due to the temperature profile or pH-values of intermediates, the preservative might be added at the end of the production. In this case it has to be taken care that no unpreserved material is stored for more than only a few hours, e.g. not over night.

Ease of use in production. An important feature of each raw material in the paint production is the user friendly easy handling making formulated biocides a necessity. Safe, free flowing pumpable, non-flammable liquids with no aggressive odors are preferred. A very important example is formaldehyde, which is as such a very aggressive, toxic substance, which can be replaced advantageously by reaction products with amines, alcohols or amides.

For example to store a 37 % formaline solution it needs a heated tank to avoid polymerization. 1,6-dihydroxy-2,5-dioxahexane [II, 3.1.4b.] (the reaction product of ethylene glycol and paraformaldehyde with more than 46% bound formaldehyde is a storage stable clear liquid even at 0°C. Today there are formaldehyde releasers available like the tetrahydroxymethyl imidazo(4,5-d)imidazole-2,5(1H,3H)-dione [II, 3.4.6.], which is a reaction product of glyoxal, urea and formaldehyde, with almost no odor and a controlled release of free formaldehyde in the paint.

Kill rate of preservatives. In principle all the bacteria, yeast and moulds commonly living in the environment can be found in contaminated emulsion paints. A very big difference to other applications of antimicrobials is the demand of total control of the broad spectrum of microbes. There is no accepted limit of microbial counts in coating materials. Some paint producers ask in their specification for raw materials for "no bacteria detectable by the means of a ready to use germ-tester". If slow killing bactericides are added in production, the time between production and test of surviving germs should be long enough to give the preservative a chance to act. It was found in practice that paints with a CIT-based preservative were claimed to be spoiled while a control of the microbial count some days later showed zero bacteria counts.

Of course the kill velocity of a microbiocide is depending strongly on the dosage. So a direct comparison of different active ingredients is not straight forward. It is best to compare standard dosages. It is demonstrated in laboratory tests that the preservatives containing only CIT/MIT as active ingredients are slowly acting while reaction products of formaldehyde with alcohols, amines and amides give a high kill rate more quickly. Sometimes a quick action is necessary to avoid enzyme formation, which can cause viscosity loss by degradation of cellulose derivatives even after the bacteria died out in the material.

On the other hand it was found that paints containing spore formers which were controlled by a stable preservative neither did change physical parameters over month storage times nor developed any bad smells.

CIT/MIT-based in-can-preservatives are today's used standard systems (Lindner, 1998; Lindner, 2001). Characteristics are the very low concentrations of active ingredients used in the products. The actual necessary concentration of the CIT in a paint might be as low as 10 mg/kg approx., based on long years experience. With the known instability against hydrolysis, reducing agents, other reactive species and the slow action against some microorganisms the main issues to improve a preservation system based on CIT/MIT are already addressed. The CIT/MIT is acting by reaction with essential molecules in the bacteria's live cycle. In competition to this useful reactions with the microbial cells to inhibit growth CIT/MIT is reacting with other constituents in the coating material. Thus a part of the CIT/MIT is used up without any effect. To ensure product quality it is necessary to prove that the CIT/MIT has fulfilled his job before it is destroyed or otherwise is complemented by a second more stable active ingredient. In the best of the cases a second microbiocide stabilizes the CIT/MIT in the matrix.

Possible scenarios are:

1. the CIT/MIT kills all present microorganisms in closed container: no microbial problem is expected unless the container is not re-closed after partial use-up.
2. the action of CIT/MIT is too slow to prevent microbes from producing extracellular enzymes which cause viscosity loss in the paint. The can is sterile while opened but the material lost its function. Addition of an enzyme blocker could improve the situation or use of a quicker acting microbicide, like a reaction product of formaldehyde with an alcohol or amide.
3. CIT is destroyed quicker than it acts against the microbes. Either use of different chemicals like BIT or formaldehyde releasers, respectively, or combination with stabilizing, quick acting microbicides are indicated.

Stabilization of CIT/MIT in coatings material. It is easily demonstrated by HPLC analysis of CIT in paints at different pH-value as well as in buffer solutions, that just at pH - values above 8 the hydrolysis is strongly accelerated. While at 30°C and pH = 8.5 the half time value of CIT is counted in month, at 40°C and pH = 9.5 after only 1 day not more than half of the dosed CIT survived.

Below pH = 8, at normal temperature conditions, the hydrolysis is negligible. To improve the performance of the preservation it is of high importance to control carefully the pH-value in every production step; especially alkaline pre-batches are to be avoided. Other reactive minor components in the matrix could be reducers like sulphite, amines or other nucleophiles. Even traces from former production steps can be enough to destroy the CIT.

It is long known that formulated water based preservatives containing CIT must be stabilized against decomposition. Effective stabilizers are molecules able to catch nucleophiles and reducing agents. Copper salts are a famous example. In application concentration the stabilization by formaldehyde reaction products is the most effective trick. A very efficient example is the 1,6-dihydroxy-2,5-dioxahexane. Today optimized combined preservatives are commercially available with excellent handling properties.

Acceleration of CIT kill-rate. To boost the kill rate of isothiazolinones the combination with the O-methylol or N-methylol compounds is an interesting strategy. Of course an increase of the dosage would speed-up the kill rate, too, but much less effective.

Similar effects can be achieved by combination of isothiazolinones with Bronopol.

Amine reaction products with formaldehyde like for example 4,4-dimethyloxazolidine (II, 3.3.10.) or 1,3,5-trisubstituted hexahydrotriazine (II, 3.3.18.) show high kill speed as well.

Alternatives to CIT/MIT. In respect to the increasing regulatory pressure in Europe, the pressure caused by the German Environmental Label "Blue Angel" RAL UZ102 as well as a guideline of the German Paint Producer Association (VdL-Guideline 01), the skin sensitization properties, the instability and other downsides of the CIT/MIT based preservatives there is an interest in alternatives (Eichstädt, D., 2001).

The already discussed O-methylol and N-methylol compounds demonstrate an important potential for the reduction of absolute CIT/MIT-concentrations. Their limits are the liberation of formaldehyde in water based matrices (Bagda, 1998).

It could be demonstrated that in emulsion paints the addition of formaldehyde releasers, in limited concentration of providing not more than 100 mg/kg of free formaldehyde in the can, did not split off formaldehyde into the indoor air above the recommended limits of the German BfR for living rooms (Bagda, 1997). A special advantage of formaldehyde is the high volatility. The formaldehyde evaporates within the first days after application and poses no risk for the end user of the room, while the emissions while painted are below worker's exposure thresholds set up by international standards. (Colon, 1998). In the USA the 100 ppb OSHA limit,

Table 3 Degradation of CIT in paint at different temperature and pH-value

Storage days:		1	2	7	14
pH-value	Temp °C	% CIT recovered			
8.5	30	98	95	86	77
9		86	77	59	32
9.5		77	55	14	0
8.5		86	80	45	18
9	40	70	48	2	0
9.5		34	9	0	0

described in 29CFR 1910.1048 "OSHA revised Standard for Occupational Exposure to Formaldehyde", Occupational Safety and Health Administration, 1992, is not exceeded even if 0.2 % of 4,4-dimethyloxazolidine with 30% total formaldehyde is applied.

To improve the activity spectrum of the formaldehyde releasers they can be complemented with 3-Iodopropylbutylcarbamate [II, 11.1.] (IPBC), BIT or MIT.

Amine based formaldehyde releasers, for example 4,4-Dimethyloxazolidine or 1,3,5-Trishydroxyethylhexahydrotriazine, are a class of bactericides, which should be considered as cost effective alternatives to the isothiazolinones. The products are stable in alkaline emulsion paints and liberate formaldehyde only slowly.

Bronopol has limited use in paints due to hydrolysis at pH-values beyond 8. If the coating material can be adjusted to this pH Bronopol is a valuable bactericide to be considered.

As alternatives to the CIT/MIT-based preservatives MIT or BIT combined with MIT are of growing interest in Western Europe, although they have to be applied in higher concentrations, and in consequence cause an increase in preservation costs. However, they are providing formaldehyde-free, organo-chlorine-free options for emission-free interior coatings. An additional benefit is the low-reactivity of the system and pH-stability up to 10.

Surface active compounds are often effective to potentiate the activity of the microbicides. It is observed that the lower the content of volatile organic carbon in a paint the more important is the formulation of a preservative to transport the active ingredient to the location within the multiphase system where it is needed. Frequently, an optimum balance of membrane-activity and reactivity of the second active ingredient against microorganisms in complex matrices has to be found. It can be demonstrated that surface active compounds [II, 18.] like e.g. quaternary ammonium salts, fatty amine derivatives, ethoxylated alcohols or dodecylbenzene sulfonic acid potentiates the activity of bactericides like BIT, MIT.

Vapor phase activity. Vapor phase activity is an important feature of paint preservatives. Especially formaldehyde donors, but chloroacetamide and CIT as well, exhibit vapour phase activity and preserve condensed water under the lid of the containers. Switching to the BIT or/and MIT-based preservatives need a modification of plant and production hygiene. To counteract microbial growth in condensed water in production units and the cans an overlay with a 1 to 2 per cent solution of a preservative containing a formaldehyde donor might be the method of choice. To produce paints with no volatile preservative it is mandatory to have a stringent hygiene concept in operation. For example all tanks and equipment should be closed by bubbler, containing a quick acting preservative like a combined CIT/MIT plus formaldehyde releaser.

It may be possible that in some production regimes new concepts of combining a slow acting but stable active ingredient for in-can preservation with a very fast but unstable "sacrifice" microbicide like 2,2-dibromo-2-cyano-acetamide (DBNPA) [II, 17.5.] to eradicate existing microbial burden from raw materials will gain importance.

Dosage of in-can preservatives. Paint preservatives are typically formulated products to allow user-friendly handling as additives in the production. Regulatory limits will further enhance the trend to more complex combined products. Combination of active ingredients is not only a tool to broaden spectrum of activity against the hazardous variety of paint spoiling microorganisms but to fine tune better the ecological and toxicological side effects as well. To make handling as easy as possible the concentration of active ingredients in the formulated products is chosen for an addition rate of 0.05 to 0.3%.

As rule of thumb the following concentrations (mg/l) of active ingredients are recommended to be used:

CIT/MIT 3:1	15 to 30
MIT	50 to 200
BIT	75 to 400
Formaldehyde from releasers	60 to 800
Chloroacetamide	900 to 2000
Bronopol	50 to 250

The lower concentration of the range is only sufficient in combined preservatives using at least 2 of the mentioned active ingredients. The higher the price of the preservative the more beneficial is a careful in-use optimization of the dosage. Optimization of preservation in paints needs a very different approach than for physical parameters because Gaussian statistics are not applicable. An optimized preservation keeps safely the microbes at the bay even under the most unfavorable production and storage conditions expected. Too low a concentration leads to adaption or selection of microbes, after a time of false sense of security the paint might be spoilt. Overdosing results in bad economics, and higher sensitization risks.

Table 4 Paint in-can preservation standards (www.oecd.org, 2000, Bagda, E., 1998)

Standard	Title	Comments
ASTM D2574	Standard test method for resistance of emulsion paints in the container to attack by microorganisms	Matrix dependant, challenge by spoilt blank containing <i>Pseudomonas aeruginosa</i>
ASTM D4783	Test method for resistance of adhesives preparations in container to attack by bacteria, yeast and fungi	• for adhesives, can be applied to water based paints • challenge with bacteria, yeasts, fungi
IBRG - Paints Project Group (draft – phase VII)	Method of test for paints – Assessment of resistance to bacterial growth in the can	Four challenges with mixed bacteria. Speed of kill estimation

5.14.3.3 Test methods

Test methods for the evaluation of a paint preservative have to be conducted in a tiered approach as no single test can model the real life situation. International working groups like the International Biodeterioration Research Group (IBRG) are working constantly on development of laboratory methods to keep up-to-date with paint development. (Lunenburg-Duindam, 2000). Besides the IBRG-method the OECD collated only three existing standard test methods for the evaluation of paint preservation (www.oecd.org).

The paint preservation tests follow the principles of a multiple challenge test. The test parameters, like choice of microorganisms, inoculation count, number of challenges, to name but a few, have to be adjusted to the test goal and pass-fail criteria are depending on requirements. In general it is not necessary to achieve quick bacterial count reduction. It seems to be more important to demonstrate the control of multi challenges. The test principle comprises the following steps:

- Incorporation of the preservative
- Pre-treatment to simulate storage conditions
- Sterility check to determine initial microbiological status
- Challenge by inoculating bacteria
- Incubation to give the microbicide time to act
- Recovery of surviving or growing bacteria from the paint

For practical purposes it is very important to check the viability of microorganisms found to cause actual problems in the paint under consideration. It is well known, that bacteria thriving in a paint might have very different resistance to microbicides than the counterpart from a culture collection. Recognition of the trouble-maker and testing with adjusted parameters is the day-to-day work of microbiological service laboratories in the industry. It is well accepted that isolates from spoilt paints can lose their special survival features if passed only a few times via an agar-nutrient. That is why the specialist uses spoilt paints as inoculum directly in these types of challenge tests. ASTM D2574 tries to imitate the adaption of microorganisms to a substrate by artificial spoilage of the blank and usage of this grown blank for challenging the test material.

It became almost common practice to check chemical degradation of the active ingredients by HPLC-techniques in parallel to the microbiocidal challenge test in cases unstable microbicides were applied.

Analytical monitoring of in-can preservatives active ingredients. After the coating is applied to walls the in-can preservative has done its job and has no further function. The whereabouts of the microbicidal molecules might be of interest. Either the molecule is fixed in the paint film or evaporates with the water, respectively. Ideally, a preservative would be consumed in the can and could be “switched-off” while the paint is coated. Especially for interior coatings the depletion behavior of the residual active ingredients is of increasing concern. The evaporation of formaldehyde from paints was evaluated to be acceptable if low concentrations of a formaldehyde releaser are used as co-biocide (Bagda, 1997; Colon, 1998), whereas the isothiazolinone emissions get more a matter of concern. In the recent literature some cases of allergic reactions to CIT/MIT emitted from interior paints via air contacts are described. (Hausen, 1999; Niederer, 1999). From the data available it might be concluded that CIT/MIT behaves like to typical semi-volatile organic compound (SVOC) in paint and gives a low but long lasting emission into the room air. As the isothiazolinones are known sensitizers the exposition of room inhabitants via air and dust should be as low as possible. That is why relatively unstable biocides might have a benefit in this respect and concepts to support the long-term in-can preservative by a fast acting but unstable molecule like Bronopol to eradicate the main bio-burden immediately after production might be worth to be considered.

5.14.3.4 Plant and production hygiene

As distinct as most other physical or chemical parameters in production, which can be controlled immediately, the effects of microbial contamination may not become apparent until it is too late to take efficient actions to keep the product in specification.

As already described earlier, spots of contaminated material in the production process are a main source of trouble caused by micro-organisms in the plant.

Unlike the original contamination, coming from the raw materials or the water, the secondary infection by spoilt materials in the equipment is much less easy to control by the standard in-can-preservation.

In all water based processes the recycling and maintenance of water is an important microbiological issue.

Due to the increasing regulatory pressure on the use of in-can preservatives opposing the trend that the coatings systems are becoming more susceptible to microbial spoilage, plant hygiene control is of constantly growing importance for the delivery of high quality coating products to the market. Waste reduction and recycling is another factor challenging the microbiological quality of a paint production. Washing liquids are often reused and stored between applications.

In principle, the preservative should protect the coatings from microbial spoilage in storage and transport, and not compensate for faults in production.

Microbiological quality of raw materials. A well controlled production should have established a microbiological specification system for critical raw materials and should follow written documented hygiene plans. Not specific to paint production is the importance of well trained and committed personnel. A hygiene plan describes what, by which means, when, how often and by whom cleaning and disinfection steps ought to be carried out. A well maintained clean plant makes it easier to establish a good hygiene practice in production. It is essential to use raw materials containing micro-organisms as few as possible. As a rule the principle “first-in – first out” should be applied to all storage operations. It is recommended to check frequently water containing raw materials or materials which are suspected to contain spore forming microorganisms after establishment of maximum acceptable counts. In practice this can be done by the paint producer himself using dip-slides or streaking samples of the material on ready-to-use agar-plates in petri-dishes by sterile swabs. If a plant manager is involved directly in establishing a hygiene regime, it is often quickly find out the critical points on which it is worth-while to focus further control actions. Identification of critical materials and production points is very helpful to optimize microbiological hygiene plans and controls.

In practice, the most critical raw material might be the water, especially if dwell water is used or water purification units are not well maintained. Polymer dispersions should be observed while stored in tanks. Overlay by an approx. 1 to 2% preservative solution containing a formaldehyde releaser to protect condense water via the gas phase is a very efficient measurement to keep the polymer dispersions and the tank in good condition. The same procedure helps to keep fresh finished goods, while stored in tanks. A lot of good ideas can be transferred from other industries producing water based materials, like e.g. cosmetics, food, adhesives (Wallhaeusser, 1995).

Industrial coatings. Main microbiological issue in the industrial coatings industry is to keep the application process under control: For example in electrophoretical coating of car bodies today, the filler is water based and this bathes are prone to bacterial attack causing coating defects as well as problems in the plant. The microbiological situation is getting even worse by the out-phasing of heavy metal electrolytes. Hygiene plans, control and use of well adapted post-added formulated preservatives are essential. Similar scenarios can be found in industrial processes for furniture coating.

5.14.5 Film preservation (dry film protection)

After a coating material is applied on a substrate the in-can preservation loses its function. Nevertheless micro-organisms, ubiquitous in the biosphere, invade all new natural and artificial surfaces, e.g. coatings, as soon as the living conditions are in their favor.

As humidity is a presupposition for the proliferation of micro-organisms, microbial defacement of coatings is common in exterior environments all over the world. However, fungi or yeast can also thrive on interior coatings, if one does not counteract by keeping the walls dry. In particular endangered are surface coatings in food production plants, like dairies, cheese dairies, breweries, in abattoirs, in warehouse and production units of the leather and textile industry, to name but a few. In domestic dwellings mold incidents occur mainly in cellars, bathrooms and in kitchens. In living and bedrooms mold is found frequently at walls where surface conditions allows water to condensate i.e. behind furniture, under windows where the surface temperature is lower due to heat transfer through not well isolated walls. In tropical countries mold problems are potentiated as growth conditions are very favorable in as well interior and exterior situations. As heating and cooling of houses is a major

factor for world's energy consumption and air pollution, the improvement of house insulation is of utmost importance today and in future. As a side effect the conditions for microbial growth on interior and exterior coatings are developing to the more favorable side.

5.14.5.1 *Microbial defacement of interior coatings*

The presence of microorganisms on a paint film is undesirable not only because of discoloration and disfigurement but also an increasing interest in the quality of air inside the buildings is discussed. Indoor air quality has a profound effect on both the performance and health of the population, since people spend most of their time indoors. Due to the tendency of decreasing air exchange in buildings, concentration of all types of air pollutants is generally significantly higher indoors than outdoors. Beside the work undertaken to identify chemicals like carbon monoxide, nitrogen oxides, ozone, volatile organic carbons, but also antimicrobials from coatings, there is a microbiological aspect of indoor air quality. Many scientists have realized that in the last years complex issues summarized under "sick-building-syndrom" or "building-related-illness" discussions are focussing more on biological particles either free floating or adhered to house dust. It is more recognized that fungal spores and bacteria may be allergens and are frequently causing health problems (Reiss, 1986). Recognizing spore sources caused by mold growth on coatings is today integral part of the investigation of air borne pollutants in interior situations. The count of air borne spores is found to be significantly higher in damp rooms with visible mold growth on walls. As microorganisms are undemanding in nutrients the limiting factor for growth indoors is the humidity. Tighter house insulation to save energy and wrong ventilation behavior of inhabitants together with construction deficits leading to local condensation of water on the coatings are the cause for fungal growth on coatings and high spore counts in indoor air. It was investigated in Europe that damp rooms supporting mold growth may cause serious health damage. It turned out that mold fungi are significantly more often responsible for health deficits than chemical emissions like solvents, formaldehyde, wood preservatives or other biocides (Senkpiel, 1994). The effects are described to be infections and allergic reactions. The symptoms are skin rashes, heart palpitations, respiratory problems, headaches and chronic fatigue. It has to be noted that even dead cells or fragments of spores may cause allergic reactions. Intoxication by mycotoxins may occur as well. Some of the mycotoxins from *Aspergillus*, *Fusarium*, *Paecilomyces* and *Stachybotrys* are well known cancerogens. Mold fungi are producers of a variety of microbial volatile organic compounds (MVOC), causing the typical mold odor in damp rooms. MVOC are a complex mixture of alcohols, ketones, aldehydes, sulfur compounds, terpenes and aromatics. Their analysis can be used as an indicator for the mold growth. Presence of these molecules are correlated with the unspecific health problems related to the sick-building-syndrom. Fungi growing on interior coatings are seeded by spores from the outdoor air, which are entering the rooms by ventilation. In living rooms the soil in flower pots is a source of xerotolerant *Aspergillus* species. In bath rooms higher temperatures and air humidity is found and a shift to fungi with high urease activity is observed, *Cladosporium* *chaetosporum* and *Aspergillus* are the common species. The spores have only a chance to adhere and to germinate on interior coatings if the surface is humid. The different conditions at interior walls select the growth of certain fungi species, like *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Stachybotrys* sp. which are found more frequently indoors than on exterior coatings in Europe. *Alternaria* sp. and *Cladosporium* sp. are growing on interior and exterior paints as well. In Europe the highest spore counts indoors are found in November and December due to heating and low air exchange. In other parts of the world with different climatic conditions and constructions the spectrum of identified colonizers is often shifted, although the air borne microorganisms and spores are travelling the world. It can be stated that species growing quickly under the conditions found, tend to override other mold. Most frequently found fungi on interior coatings are more xerotolerant species of

- *Aspergillus* sp.
- *Penicillium* sp.
- *Cladosporium* sp.
- *Fusarium* sp.
- *Alternaria* sp.
- *Chaetomium* sp.
- *Aureobasidium* sp.
- *Stachybotrys* sp.
- *Mucor* sp.
- *Trichoderma* sp.

Yeasts are found only in rooms with high humidity like bathrooms:

- *Candida* sp.
- *Rhodotorula* sp.

To prevent mold growth on interior coatings the relative air humidity should not exceed 65%, of course this must be true for all parts of the room, even for the area behind the furniture and at relatively cold walls.

In Europe and the USA air conditioning or correct manual ventilation should be sufficient to avoid mold growth in living rooms. Due to extreme low air exchange rates in modern "low energy houses" of below 0.5 m³ air exchanges per hour the air humidity rises if the ventilation techniques are not adapted. Probably it needs extended training courses in manual ventilation techniques to convince inhabitants to transport out the estimated 15 l per day of water produced per person by cooking, showering and breathing or to get the humidity transfer done by artificial air conditioning. In general, film preservatives should not be used for interior coatings in living rooms. In tropical climates (and) under certain circumstances it might be of a lower health risk to apply a film fungicide than to leave the people living in damp moldy rooms (Hunter, 1989).

Coatings in food production units can be supported to comply with hygiene standards if carefully designed and if film preservatives are used. Primary infestations are quite easily kept at bay. The susceptibility of the surface can be reduced by choosing paint ingredients with inherent resistance to be degraded by microorganisms. For example cellulose derivatives as thickeners should be avoided, vinylacetate binders may contribute acetates by hydrolysis into a coating, which are good nutrients for microbes. Easily biodegradable plasticizers like the citrates should be avoided. A controversial discussion takes place about the optimum hydrophobicity of coatings to be the least supporting for microbial growth. The situation is not clear cut as it is known from biofilm formation that microbes tend to adhere preferentially on hydrophobic surfaces due to van-der-Waals and electrostatic interactions (Eckhart, 1996). It appears to be important that the stickiness of the surface is kept low. Water uptake of the binder should be low as well. The binder should form hard, non-sticky surfaces. Hard smooth surfaces makes settlement by microbes difficult. Mold growth is impeded by higher pH-values, but surface pH is becoming neutral quite fast by carbonatization. Except zinc oxide and borates the pigments and fillers do not contribute to susceptibility. Incorporation of a film preservative can easily prevent spore germination, if the active ingredients are available at the surface.

Much more difficult to control is the secondary growth of microbes on impurities sticking at the coating surface. Secondary growth is characterized by utilization of nutrients from the dirt layer, which may have altered the surface characteristics of a coating completely. The spectrum of microbes thriving on these dirt layers are depending on the industry the coated rooms are used in. In breweries *Aspergillus niger* and *Cephalosporium* may be the dominant species while in the cotton processing textile industry *Chaetomium* and *Stemphylium* sp. with high cellulase activity prevail. The mold are not thriving directly on the coating material but on a layer of dirt. Similar situations are found on biofilms, where secondary invaders are using the organic material formed by the original colonizers and thrive on this layer. A fungicide incorporated in the coating material has to migrate into the dirt layer or the biofilm to prevent secondary fungal growth. Of course, in this scenario, this fungicidal molecule is intended to be depleted from the coating and the action can only last until the reservoir is exhausted. The disadvantage of the migrating fungicides is the contamination of the environment. On the other hand all attempts to design polymeric non-migrating film preservatives proof to be in-effective, which is what has to be assumed taking the under-laying principles under consideration.

The general requirements for interior coatings film preservatives are similar to the one for in-can preservatives already mentioned, but some of the specific ones are different. As the application conditions might be very different in interior scenarios a single preferred film fungicide can not be favored. While pigment like substances, such as zinc oxide or barium meta-borate [II, 8.2.1b.], are excellent in damp storage rooms, where slowly growing fungi have to be controlled, this could be very different in wet food processing areas, where the walls are physically cleaned by a water jet day by day and leaching of the fungicidal active is the critical point. For instance in dusty grain processing areas secondary growth of mold on the deposit layers is the most important threat and can not be controlled but by a fungicide with sufficient mobility.

Requirements for interior coatings film preservatives:

- Broad spectrum of activity, the product should be active against bacteria, yeast, fungi known to be spoilage organisms on paint films
- Cost effectiveness
- Stable under pH conditions of the paint (8 to 9.5, or even up to 12 in silicate systems)
- Long lasting activity by balanced migration in the coating film
- No discoloration
- No viscosity influence
- Low odor
- Low water solubility
- High partition coefficient (pK_{ow}) between organic materials and water
- Low leachability, low volatility
- Low toxicity
- Easy handling in the plant

- A good ecotoxicological profile
- Regulatory compliance

As the property of a film preservation is defined by the interaction of the microbicidal agents with the coating in service, the optimization should be done on the complete system. Although carefully designed microbiological laboratory tests can help to select film preservatives the “gold standard” test are field studies. As no laboratory test can simulate the specific scenario in respect of the specific conditions, like e.g. soiling, microbial consortia, succession etc.

5.14.5.2 Interior film preservatives

The most important fungicides used today in interior coatings are Iodo-propinyl-butyl-carbamate (IPBC) [II, 11.1.], Carbendazim (BCM) [II, 11.4.], Octylisothiazolinone (OIT) [II, 15.4.], Dichloro-octylisothiazolinone (DCOIT) [II, 15.5.], Zinc Pyrithione (ZPT) [II, 13.1.3b.], Zinc dimethyldithiocarbamate (ZDTC) [II, 11.11.3.], Thiuram [II, 11.13.1.], Barium meta-borate [II, 8.2.1b.]. Carefully designed formulated film preservatives based on one or more of these active ingredients are world wide available. Regulatory restrictions are to be considered in the highly regulated markets like the USA and the future Europe.

Hygienic coatings. A special issue are the so-called hygienic surfaces, which refers to a concept of surface equipment against bacterial infestation in the medical and institutional sectors, food industry and similar areas, by using a microbial control mechanism going out from the coating. A fundamental difference to conventional film preservatives is the intended antimicrobial action by the coating, while film preservatives typically are used to protect the coating itself. Besides the use of bactericides like e.g. Triclosan [II, 7.6.1.], other technologies are under development not using conventional microbicides. Easy to clean low energy surfaces, photocatalytically activated systems, repellents are options to name but a few. As the requirements for hygienic coatings are very different from film protected paints new microbiological test methods have to be developed. (www.ibrg.org) These new test methods have to reflect the control of microbes coming into contact with the surface and must be different from standard long term film preservative tests and kill-rate disinfection tests at the other side. Development of microbiological tests for hygienic coatings should be done in close relation to other surface related materials like plastics, ceramics and metals.

5.14.5.3 Microbial defacement of exterior coatings

Exterior surfaces exposed to the environment are in contact with air borne microorganisms. Microorganism conquer all accessible surfaces in the biosphere, as they are undemanding and adaptable. Newly built surfaces as for example islands formed by volcanic activity are invaded after only a few days by microorganisms. No open body of freshwater remains devoid of bacteria and algae for much longer than the first gust of wind. Bacteria, fungi, algae and lichens are often found in succession and at the same time living in consortia. Atmospheric pollution observed in industrial areas all over the world tend to suppress the growth of a variety of microbes and therefore the most resistant ones are dominating here.

Especially in summer times the air borne fungal spore concentration can be as high as 10^5 per cubic meter, in direct neighborhood of freshly harvested cereal fields even much higher. Air borne microbial spores are extremely mobile and can be found all over the world. Spores are coming from the soil and the plants and like to adhere to dust, soot and pollen. Sources are the rotting biomass on soil where a consortium of microorganisms are deteriorating the organic material produced by plants, animals and other microbes.

It can be summarized that the following genera are found by air sampling most frequently all over the world:

- Cladosporium
- Alternaria
- Aureobasidium
- Epicoccum
- Ulocladium
- Aspergillus
- Penicillium
- Paecilomyces
- Phoma
- Stemphylium
- Fusarium
- Rhizopus

It does not surprise that these species are most frequently isolated from exterior coatings as well. Black spores are insensitive to UV-light and this helps these so-called black mold to dominate in exterior situations. Air

sampling all over the world revealed, that the dark colored spores of *Cladosporium*, *Aureobasidium* and *Alternaria* are most frequently found. Less pigmented spores are eradicated by sun-radiation and find less chances to settle on coating materials.

Algal cells are found in water or on terrestrial ground, on stones and on the bark of trees. Algae are transported by air. Predominant species found in air are those thriving on solid surfaces. This is not surprising as the algal cells are torn off by wind from drying bark, plant surfaces, or soil, respectively. Algae can survive dry periods for extremely long times. That is why algae are found in the air of very different geographic regions in the world (Baumann, 1979). Typical species found in investigations identifying air borne algal cells in America, Asia and Europe are

- *Klebsormidium*
- *Pleurococcus*
- *Chlorella*
- *Chlorococcum*
- *Scenedesmus*
- *Stichococcus* species

and cyano-bacteria (blue green algae)

- *Anabaena*
- *Chroococcus*
- *Gleocapsa*
- *Nostoc*
- *Oscillatoria*
- *Phormidium*
- *Scytonema*
- *Tribonema* species

which are frequently found accompanied by local predominant species. In South East Asia

- *Trentepohlia* species

are spread widely (Wee, 1980; Wee, 1982). Cosmopolitan species are found to be transported by the wind over intercontinental distances Coleman (1983) stated, that air sampling of algae is remarkably repeatable, and that samples taken in Michigan, Texas and Hawaii are not particularly different.

On exterior coatings air borne microorganisms might find conditions to adhere and to thrive. Prerequisite for occupation by microorganisms is the presence of humidity, at least for some time a year. Spores can survive long dry times on surfaces and start growing very quickly, provided the growth conditions are turning in favor for growth. Paints and stucco containing some organic materials and minerals in combination with humidity, and for algae additional light, are good substrates for biofilm formation. In general different microorganisms are settling on the surfaces, frequently symbiosis and a succession of several different microbe species is observed. Surfaces colonized by a single species are not often observed. Microbes are using symbiotically their different kind of metabolism of their respect partner. Once a biofilm is formed, the original properties of the coating are changed and further predators might find altered living conditions. Biofilms retain humidity and offer this to secondary invaders as well as new organic nutrients. It is often observed on colonized walls, that areas are not equally covered, and an existing local biofilm is accelerating further growth. Auto-acceleration produces typical growth-patterns at walls which are much more disturbing for the human eye than an even color change. Similar patterns are always observed on panels from exterior exposure studies.

Especially on wooden substrate fungi are frequently found to penetrate the paint film, starting from the substrate. A similar observation can be made, if molded surfaces are re-painted without eradication of the present microbes. Micro-cracks allow fungi to penetrate the coating and to grow beneath on the substrate. The most common scenario of molded surfaces is the secondary growth on adhering organic soil particles, which provide a rich substrate for fungi. After first invaders like algae start thriving fungi are second to use the newly conditioned surface as a substrate.. These mechanisms are playing against a theoretical strategy to avoid colonization by rendering the coating material non-degradable or to use polymeric non-migrating active ingredients.

The primary consequence of fungal and algal growth on house walls is discoloration and disfigurement. Discoloration can vary from gray, black, orange-red, brown to green. Hyphae of fungi can extend into paint films and extracellular enzymes are able to degrade polymeric binders. Graying of a paint film is not necessarily an indication for microbial defacement, because dirt particles are sticking quickly at surfaces by van-de-Waals interactions. In the first adherence step living particles are not different from dust.

As a secondary effect the paint film might be attacked, cracks are formed and the protective function of the coating for the substrate might be lost. Algal population on coatings does not destroy the material but it can be

suggested that the change of the surface properties like humidity retention, loss of hydrophobicity and the tension produced by temperature alterations might cause micro-cracks. Bacterial growth is not visible on houses, but seems to be influencing colonization by other microorganisms by conditioning the surfaces.

It is apparent that invasion of an exterior coating is following a similar mechanism as other biofilm formation. As microorganisms use hydrophobic van-der-Waals interactions as mechanism to adhere, hydrophobic surfaces attract more airborne spores. Surface water is always polluted by organic material from the environment and quickly a conditioning film is formed, in a next step airborne microbes start germinating (Flemming, H.-C., 1995). See also chapter 5.1.

Wetting tendency, polarity, thermoplastic behavior, stickiness, porosity and surface structure are factors influencing the soiling of a coating as well as the tendency to microbial defacement. Binder, PVC, extenders, pigments as well as the additives like coalescent and thickener do influence the behavior of coatings in exterior situations. Chalking is an efficient mechanism of constant cleaning of a coating, but this is not an option for high quality paints.

Of course different substrates to be commonly used on house walls all over the world and different climatic conditions have to be considered.

Non-preserved coatings, no matter they are classified as mineral -, styrene acrylic -, or silicon bound stucco, or paint finishes, are prone to microbial defacement.

In the USA wooden sidings are common covering about 40% of all homes. In general the sidings are coated with low PVC coats based on acrylics and alkyds. The chemically drying alkyd paints used to contain heavy metal drying catalysts, like (informer times) lead, providing substantial algicidal action. The physically drying acrylics are less resistant to algal growth and this is why it might appear that algae are more spreading on house walls in the USA and a need for algicides was only emerging recently. Micro-cracks in paint films allow *Aureobasidium* spores to penetrate into the under-laying wooden substrates and discolor the wood. While in Scandinavia solvent borne alkyds were used, no incidences with the fungus *Dachromyces* species were found, but houses coated with more modern water based acrylic paints were attacked. On painted concrete walls of flats with high initial pH in the tropical South East Asia algae find it very easy to thrive after only very short time in service.

In Germany due to high energy prices and governmental regulations the exterior insulation and finish systems (EIFS) became a standard for flats, which were previously built in the conventional manner with brick walls. The energy transmission via the wall is controlled by a multi-layered construction fixed at the outside. The inner layer, directly fixed to the wall either mechanically or by an adhesive, is an insulation board made from foamed polystyrene or mineral foam, respectively. The insulation sheet can be thick up to 18 cm. The next layer is a polymer containing thick coat with a plastic reinforcement mesh embedded. This layer is the basis for the finish coating, a stucco. The stucco can be either mineral or polymer dispersion bound. To increase hydrophobicity silicones are frequently incorporated. These construction method is the most economic approach to support the fulfill of the demands of the 1995 Kyoto-protocol to decrease carbon dioxide emissions from heating and air conditioned homes significantly. An alternative construction method used for small homes is to use insulating light porous bricks to build the walls (monolithically insulated houses).

The heat insulation of walls has a significant impact to the humidity conditions on the coating surfaces, as at night the surface cools down by IR-radiation into space below the dew point of the atmosphere humidity. Due to the insulation no heat is transported from the inside of the house to the surface and the surface stays damp for longer times. This provides good growing conditions for algae and mold. Further factors discussed to explain increasing microbial problems on EIFS in Europe are not proven but are appearing reasonable (Grochal, P., 2001):

Improving the air pollution situation might be beneficial for algae and fungi, growing on walls. The increasing carbon dioxide concentration and the warmer climate ("global warming") promotes algal growth as well. Change in methods of agricultural activity might be important for growth conditions as well. Microbe nutrients are introduced in the air by fertilizers. The tendency to decrease pesticide use or to use more selective pesticides in European agriculture might have an influence on the ecology on walls, as well.

Architecture can help to keep walls dry by the right construction of roofs and gutters, balconies, and by avoiding conducting areas. Trees and bushes should be planted only in a reasonable distance to the house to avoid organic debris.

Today, it is state of the art to add a film preservative to the finish coating material to keep microbial defacement at bay. Objects, known to be situated in regions with high fungal or algal activity should be coated with especially film-protected coatings. Micro-climatical differences are sometimes observed within distances of only a few hundred meters. While an identical coating is grown on the first building, the neighbored house might be clean for long time. This is in line with the general observation, that the direction the coating is exposed to is a salient factor for growth. In general, a broad spectrum film preservative is applied in higher concentration for object related protection. International companies are already used to apply different paint formulas for the application in different regions of the world. It is accepted that a paint applied in Texas needs to be different from the one in Florida or in Michigan.

Different film preservation requirements are also related to local painting habits. While the home owners in some South American countries are used to repaint their homes after two seasons latest, they do not need a film preservation for more than only 2 years. In Germany the thick coatings on EIFS are warranted by the producer for five years minimum, and customers expectations to have a clean wall are going even far beyond this period. Here, of course, more enthusiasm has to be put into the film preservation. In the tropical countries colonization of unprotected walls is that fast, that a very efficient broad spectrum film preservative is a must, because walls cannot be kept dry in this climate.

Detection of microbial growth on coatings. The identification of microbial defacement of coatings in exterior situations is not a trivial task and needs costly laboratory investigations for each case. Isolation and determination of a microorganism from a coating is not a proof for a causal relation to film colonization and disfigurement.

It can be suggested that many more cases of discoloration of house walls are caused by biofilms than it is anticipated in the paint industry.

In general, coatings in exterior situations behave similar to natural substrates and it is no surprise that the fungi and algae, described above, isolated from air samples all over the world, are under the main causative microorganisms found to grow on coatings.

The detection of microbial growth on walls is a multi step approach. Experts can identify macroscopic growth patterns, the next step is the sampling from the surface by sterile swabs or by an adhesive tape. It has to be made sure, that the microbes thriving in the biofilm are sampled and not only those laying as spores loosely on the surface. With a light microscope mold and algae are visible as single cells and sometimes can be classified. Cultivation in nutrient broth might be necessary to produce features, like spores, helping to identify closer the microorganisms involved in coating defacement. By doing this, it has to be kept in mind, that microbes on the coating are thriving under very different conditions than on an agar nutrient in the laboratory.

It is very important to recognize that the microorganisms isolated from coatings are generally less sensitive to microbicides than related laboratory species from culture collections. This is of importance in film preservative efficacy checks.

Mites and spiders on exterior coatings. In recent years, in several regions in Europe coated house walls are disfigured by invasion of small spiders, like *Dictyna civica* (Wicki, 1998).

The nets of the only a millimeter large spiders are sticky and adsorb soot and dust and become visible as dark spots. As the net is built from proteins, microorganisms accept them as nutrient. A reasonable counter action is not yet found to prevent invasion of *Dictyna civica*.

Exterior coating materials. In general the finish coating of exterior walls should have a low water retention and low water penetration. Easily degradable thickeners should be avoided. The soiling tendency should be minimized and a broad spectrum film preservative should have been added in production. It was assumed for long time, that highly alkaline mineral bound paints and stucco are self-resistant to surface colonization. In practice, it was found, that the alkalinity at the surface is neutralized by carbonatization within a few month. The same effect has been found on painted fresh concrete. With an extreme hydrophobation and micro-structure formation by silicone additives as a mimicry of the lotus-leave it is tried to offer spores no chance to adhere. An unsolved issue with such type of paints is the long-term stability (Born, 1999).

Table 5 Isolation of microorganisms from exterior coatings in Germany

System investigated	Fungi found	Algae found
EIFS – styrene acrylic stucco	Alternaria spec. Cladosporium spec. Less frequent: Chaetomium spec. Penicillium spec. Aspergillus spec. Yeast: Rhodotorula	Single ball- to oval-shaped green algae: Chlorococcum spec. Chlorella spec. Pleurococcus spec. Blue green algae: Gleocapsa spec. Nostoc spec.
EIFS – silicate stucco	Alternaria spec. Cladosporium spec.	Green algae: Chlorococcum spec. Chlorella spec.
Silicon paint	Alternaria spec. Cladosporium spec. Less important: Stemphylium spec.	Green algae: Chlorococcum spec. Chlorella spec.
Silicate paint	Alternaria spec. Cladosporium spec.	Blue green algae Green algae: Chlorella spec. Blue green algae

5.14.5.4 Exterior film preservatives

Adding a film preservative in production improves coating performance in service. Repainting intervals are significantly prolonged. This provides an economical and ecological value. In the ideal situations, the construction plans of the architect consider physical measures to keep the walls dry and, additionally, the coating material comes well optimized in respect of physical properties together with an appropriate film preservative. It has to be born in mind, that the stability and efficacy of the film preservative is directly related to migrating and leaching behavior of the active substances in the finished coating.

The film preservative is generally a formulated microbicidal product, adapted to the physical form of the coating material. For liquid products liquid film preservatives are preferred, for powder coating materials a powdered preservative is mandatory. Liquid film preservatives are either solutions in solvents common to the paint formulator as coalescents, like the glycol ethers e.g. dipropylene glycol methyl ether (DPM), or stable fine particle dispersions in water. Dispersions are providing an additional factor to optimize efficacy and migration behavior, respectively, the particle size distribution, which can be easily adjusted by wet-milling. The liquid film preservative should be stable under the storage conditions, no settling of particles should be observed, even if stored under varying temperature conditions. The rheology should have a Newtonian characteristic for easy metering. In the development of formulated preservatives the inert behavior in paint formulations is a main goal.

The film preservative should have the most as possible of the following properties:

- high activity against a broad variety of microbes,
- against black fungi including *Alternaria* species,
- against other mold, including blue stain like *Aureobasidium pullulans*,
- against yeast, cyano-bacteria (blue-green algae) and green algae.
- Cost effectiveness
- Stability in the wet state at the pH conditions of the coating material (8 to 9.5, or even up to 13 in silicate systems)
- No discoloration in-can and in-film on all substrates the coating material might be applied to
- Compatibility with all coating ingredients, no influence on physical-chemical properties
- Low odor
- Very low water solubility
- High partition coefficient (pK_{ow}) between organic materials and water
- Long term stability in the film (against UV-radiation, air pollutants, hydrolysis)
- Mobility sufficient to diffuse to the surface
- Low toxicity
- Easy handling like an additive in the production
- A good ecotoxicological profile in production (waste-water) and in service (non-persistent)
- Regulatory compliance

Even under most severe climatic conditions in service the stability of the film preservative active ingredients ought have to be guaranteed for a couple of years (Bravery, 1988).

While in the early times of film preservation the organometallic compounds (II.19.) used were active against all creatures, these phenylmercurials and tributyltin compounds are no longer in service in most countries of the world due to their unfavorable ecotoxic behavior. The replacement of solvent borne coatings, which were chemically drying by the catalytic activity of heavy metal salts with certain antimicrobial activity, by water based systems rendered the coatings more susceptible to microbial infestations. Today, active ingredients used world wide are in general organic molecules of molecular mass of 200 to 500, with low volatility, water solubility of 2 to 300 mg per liter, an octanol-water partition coefficient of log 2.5 to log 4, which is an indication for the right migration behavior from a polymer containing coating material to the surface (Lindner, 2000).

These physical properties appear to be a good compromise between mobility and stability in the coating film. It could be demonstrated, that molecules equally active in microbiological nutrient test systems might be very different in paint films. Unfortunately, the high development and regulatory costs are too high hurdles to consider physically optimized derivatives of existing active ingredients as better alternatives for certain coating types. Very good candidates would be the homologous 2-alkylisothiazolin-3-ones or the homologous 2-Propinyl-alkylcarbamates, respectively, offering a rather broad window of activity, while designing the alkyl part to be optimal on physical reasons.

The organic molecules used are either dissolved in the polymeric binders or are present as solid particles and this is the basis for leaching stability. Film preservatives dissolved in the polymeric binder matrix are typical slow-release systems (Heaton, 1991; Hughes, 1983). This is an explanation for the very different efficacy of a given film preservative in coatings with different binders. The currently used active substances in film preservation are rather selectively acting. The requirements on the film preservative can be best fulfilled by combination of active ingredients. Not only the broader microbiological spectrum has to be considered but also the complementary

migration properties necessary to cover all growth hazards either in the early lifetime of the coating or after long service times which might be quite different. In the early service times the coating does contain additives like thickeners, film forming agents, dispersants which have lost their functions after the coating film dried, but their presence still influence the susceptibility of the film for microbial growth. Today actually used film preservatives are carefully formulated combination compounds containing two to four active ingredients besides components, which guarantee easy handling under production conditions in a paint plant. The pool of actually used active ingredients is very limited. A coarse classification can be done into algicides and fungicides. Typical algicides are interrupting the photosynthesis of algae and do not possess significant fungicidal, or bactericidal activity. In the class of the currently used fungicides, there are found very selective ones, like the triazoles [II, 14.1.] or Carbendazim, but others like zinc pyrithione or the alkylisothiazolinones have additionally useful algicidal and bactericidal activity as well.

Solvent borne coatings can be protected against fungal growth by N-trihalomethylthio-compounds [II, 16.]. Especially solvent borne wood coatings are efficiently equipped against mold and blue stain fungi by Dichlofluanid [II, 16.5.] or Tolyfluanid [II, 16.6.]. Worse physical properties like solubility render the related alternatives Folpet, Fluorfolpet and Captan less popular. The N-trihalomethylthio-group is very sensitive to hydrolyses and the use is limited to solvent borne low PVC-systems.

Iodopropinyl-N-butylcarbamate (IPBC) found wide-spread application in both, the solvent borne and in water based systems, respectively. It is by far the most popular Iodine-organic fungicide world-wide for coatings. The upsides are the very high and broad fungicidal activity at reasonable costs against all relevant coating colonizers, including *Alternaria* species, a very good toxicological profile, authorization in highly regulated markets like Canada, USA, Scandinavia and other countries. The unstability against degradation by UV-radiation, high pH, or catalytically active metals, like e.g. cobalt, is turning into an advantage in respect of low persistence in the environment after leaching from a coating. Downside is the low stability against several paint ingredients, and UV-radiation causing yellowing in white paints. There are methods to stabilize IPBC in coatings, but these need highly selective adaption of the formulas to this active ingredient. The discoloration is usually recognized in fresh coatings, and often disappears quickly by evaporation, because iodine is involved. The algicidal activity of IPBC is very limited to a few species, and typically, IPBC should be combined with distinct algicides to form a broad spectrum film preservatives. The formulated products in the market are either solutions in typical coalescent solvents or dispersions in water. The pH-unstability limits the applicability of IPBC in water borne coatings for mineral surfaces, which are produced in general with pH-values higher than 8.5. Rather high water solubility and octanol-water partition coefficient limit the use in paint films on mineral surfaces as well, and are the factors driving the necessary dosages up.

The triazoles used in film preservatives are Tebuconazole and Propiconazole. Both were developed as crop protection agents and found secondary application as wood preservatives. Other imidazoles and triazoles from the plant protection area might be as well suited, but were not developed into the film preservation application. Besides the activity against wood destroying fungi, both molecules possess useful activity against mold and yeast. The activity of the triazoles against "black fungi" including *Alternaria* species is not very pronounced, but can be used in combination products advantageously. Triazoles do not cover *Penicillium* species. They do not provide algicidal activity.

Due to the favorable physical properties, triazoles can be formulated equally well in either solvent borne paints or water based coatings.

Interesting features are the stability against hydrolyses even at high pH-values which gives the triazoles some attraction to be used in silicate bound stuccos and paints or in coatings applied to freshly prepared concrete walls.

Table 6 Biocidal active substances for film preservatives in exterior coatings are used in different applications as well. The indicated dosage is a typical concentration in cases combined preservatives are used.

Active substance	Film preservative application	Dosage % based on paint	Further application	Typical dosage % in further applic.
Carbendazim (II, 11.4.)	Fungicide	0.1	Crop protection	0.2 g per m ²
Octylisothiazolinon (II, 15.4.)	Fungicide + algicide	0.05	Metal working fluids	0.05
IPBC (II, 11.1.)	Fungicide (+ algicide)	0.1	Cosmetics	0.02
Zinc Pyrithione (II, 13.1.3b.)	Fungicide + Algicide	0.1 to 0.5	Antidandruff Shampoo	0.5 to 1.0
Diuron (II, 10.9.)	Algicide	0.2	Herbicide; PU-catalyst	1 g per m ²
Terbutryn	Algicide	0.1	Herbicide	0.1 g per m ²
Mctt (IRGAROL 1071) (II, 20.4.)	Algicide	0.05	Antifouling ship paints	5

After improved synthesis procedures which provided colorless qualities of Carbendazim (BCM) almost free of critical impurities, the active ingredient became a work horse as fungicide for film preservation in the eighties and nineties starting from Europe. A marketing survey revealed, that 1998 in about 80% of the thick coatings for EIFS in Germany BCM was detected (Lindner, 2000). Originating from crop protection applications in cereals, Carbendazim shows extremely high activity against mold and blue stain, is almost chemically inert, has very low leachability, and extremely low vapor pressure. Even in alkaline silicate paints the molecule does not hydrolyze. On the down-side, Carbendazim does lack any bactericidal or algicidal activity, has gaps of activity against some of the most important “black fungi”, like *Alternaria*, or *Ulocladium*, and certain yeast of the genus *Candida*. Film preservatives for exterior coatings based on Carbendazim always consists of a combination with further active substances, filling in the gaps. Carbendazim provides synergistic effects with e.g. octylisothiazolinones in respect of breadth of activity and physical depletion properties, and this is why these combination preservatives provide long lasting protection at reasonable active ingredient concentrations, and in parallel with that, reasonable costs. The activity gap against *Alternaria* led to a situation in parts of Europe, where *Alternaria* species are almost the only mold species causing a threat for exterior coatings. In opposite, in the USA, where Carbendazim was registered by the Environmental Protection Agency (EPA) as a film fungicide only in 1996, *Aureobasidium pullulans* (blue stain) was the predominant fungus found on coatings (Brand, 1973). In Europe, *Aureobasidium pullulans* does not play any role on surfaces, because it is well controlled by Carbendazim. As Carbendazim has a very specific target to interact with fungi, acquired resistance might be responsible for some failures, observed in practice.

Thiabendazole (TBZ) [II, 15.9.] is another benzimidazole derivative, which could be used in a similar manner as BCM, but the relatively low performance-cost ratio limits the use.

Due to its high and broad spectrum of activity, combined with desired physical properties, 2-n-Octylisothiazolin-3-one (OIT) finds broad application in film preservatives. The combination with Carbendazim is one of the most efficient fungicide systems known. High mobility and relatively good water solubility are playing against durability in coating systems. Properly chosen binders, which are functioning as depots, are able to immobilize the OIT in a coating to provide reasonable service times.

OIT, as molecule in a compound, is worth to work on in optimization of a coating system. Improvement of the depletion characteristics from the coating is the key and can be achieved by proper selection of the organic binders. There is no rule of thumb available to predict the retention of OIT by polymer films formed from dispersions, but in general a hydrophobic styrene-acrylate dispersion provides the best slow-release characteristics, while vinyl-acetate-copolymers are less suited. The binder can be regarded as a depot former, which has to be present in the coating anyway for physical reasons, and could be used without additional costs for encapsulation. The fact of incorporation into the binder, demonstrate the necessity to develop an exterior coating as a complete system.

As regulatory costs for active substances are going up constantly, the focus of research is shifting towards the principle to get out the best from the existing molecules. Encapsulation of Octylisothiazolinones into inorganic matrices might be an alternative, to improve long term retention in a coating, and allows to reduce biocide levels added to the coating material (Gibson, 2001). Of course, the effects of encapsulation have to be counterbalanced to the additional cost of incorporation of the inert host material. As the requirements on an efficient long term encapsulation are much higher in the coating application than in the crop protection area, where the technique is applied successfully, it will be interesting to observe, whether these concepts will be viable.

As the pH-stability of OIT is only guaranteed up to 10 in homogenous systems, the incorporation into an appropriate binder or encapsulation into inorganic materials can protect the molecule against hydrolysis even in

Table 7 Leaching of BCM and OIT from different polymer dispersion films demonstrating the effect of binders on the depletion rate from coatings

Polymer	Leaching hours	BCM %	OIT %
Styrene Acrylate A	48	83	84
	96	70	72
PVA-PE-Polyvinyl-chloride-terpolymer	48	55	35
	96	32	14
PVA-PE-pressure polymer W	48	43	22
	96	24	2
Pure acrylate B	48	69	77
	96	86	54
Styrene-acrylate with wet adhesion promoter	48	67	49
	96	65	44
PVA-PE-pressure polymer C	48	86	74
	96	93	57
Styrene-acrylate Q	48	84	60
	96	69	38

Table 8 Performance of OIT in laboratory fungi challenge test according to BS 3900 G6 (Gibson, 2001). It could be demonstrated that silica-encapsulated OIT performed much better against *Alternaria alternata* than the same concentration of free OIT added

Sample	OIT level %	% fungal growth surface coverage
Silica-OIT-encapsulated	300	32
	1200	3
	4000	0
OIT (non treated)	300	85
	1200	35
	4000	3
Blank control	0	85

coating materials with a pH as high as 12. These surprising effects can be used to apply OIT even in silicate paint and stucco, or to use the respective coatings on freshly prepared concrete surfaces without damaging the activity of the biocide. As the most destructive situation for OIT is in the wet state, the storage conditions of such paints ought to be controlled carefully.

4,5-Dichloro-2-n-octylisothiazolin-3-one (DCOIT) [II, 15.5.] provides a very broad and high antimicrobial activity spectrum against fungi, yeast, some bacteria, and some algae. The efficacy against *Alternaria* species is excellent. Due to the higher hydrophobicity, compared to OIT, the water leachability from coatings is a non-issue, but volatility is even higher, and mobility in the coating in some cases might be too slow. The down-side of the molecule is the high sensibilisation risk and the inherent instability. Hydrolysis is fast at pH-values higher than 8,5, and reactivity with amines is limiting the application in alkyd bound paints.

2-n-butyl-1,2-benzisothiazolin-3-one (BBIT) [II, 15.7.] is a rather new active substance, originally developed for the fungicidal protection of plastic material. Water solubility might be too high for the application in paints, but in combination with a well chosen polymer dispersion, BBIT might be an alternative combination partner to protect thick coatings, especially on EIFS, against *Alternaria* and other black fungi.

Chlorothalonil [II, 17.19.] has some importance as a film fungicide in the USA, providing a non-leachable protection against fungi and some algae, as well. Volatility is quite high and the molecule tends to promote chalking of paints, which hinders its use in colored coatings. pH-stability is not high enough to make Chlorothalonil a useful fungicide in coatings for mineral surfaces. The mobility in low-PVC-coatings is sometimes insufficient. The high contribution to the waste water parameter AOX (adsorbable organic halogen) hinders from the use in Europe.

An even more limited application finds tetrachloro-4-methylsulfonyl-pyridine [II, 17.12.] despite good activity against coating relevant fungi and algae due to its unfavorable chemical and physical properties.

Zinc pyrithione (ZPT) [II, 13.1.3b.] is an interesting active ingredient for the film preservation with pronounced activity against "black fungi", including *Alternaria* species, yeast, some bacteria and several algae species. Admittedly in all groups of relevant microorganisms, there are some, which are not sensitive to ZPT. In general, this is why ZPT has to be used in combination with further active ingredients to keep the dosage in limits compatible with coating materials. The pigment like character causes low mobility in coatings and this downside has to be compensated by a combination partner as well. The pyrithione ligand is considerably stable, but in alkaline systems the zinc cation is displaced from the complex by calcium which renders the molecule highly water soluble and prevents its use in silicate coatings. ZPT – containing coating materials should be applied very careful on alkaline substrates like concrete walls, because by destruction of the complex, traces of heavy metal ions, especially iron, react immediately to form highly colored complexes. While ZPT is almost non-leachable from paints at pH 9, depletion is very fast at higher pH-values, due to hydrolysis of the complex.

Due to the threat to discolor with heavy metal traces the dithiocarbamates, a very effective class of bactericides, fungicides, and algicides, namely Ziram [II, 11.11.3.] and Thiuram [II, 11.13.1.], respectively, are not often considered to be used in coatings. Their application is almost limited to interior coatings in industrial environments. It has to be noticed, that the complexing activity of the dithiocarbamates is deactivating the heavy metal driers used in chemically drying alkyds.

As algal growth on house walls was often confused with fungal defacement, rather high loads of fungicides with algicidal side effects were used in the past to control the situation. The shift from solvent born alkyd coatings to water based acrylics to paint the wooden sidings of US-houses caused more algal defacement, because the heavy metal driers were quite effective algicides. In Europe it was recognized in the late seventies, that the EIFS used to save heating energy were especially prone to algal attack and pronounced algicides were introduced into the film preservation segment. The physical phenomenon of superficial energy emission from the EIFS finish coating by IR-radiation, followed by condensation of dew, explains the local climatic conditions in favor of algal growth. Today, it is recognized all over the world, that algae can be kept most efficiently at bay by special coating

additives, containing molecules, which interact with the algal photosynthesis. Some total herbicides of the dimethyl-urea or triazine class, commonly applied in crop protection, are possessing useful algicidal activity as a side effect. The first of these type of dedicated algicides – Diuron (DMCU) [II, 10.9.] – was introduced as coating film algicide in Europe in the late 1970s, after the phenylmercurials disappeared as a class of fungicides with pronounced algicidal side effect. Almost all important green and blue-green algae thriving on coatings are controlled by Diuron. Diuron is almost inert and hydrolysis is only an issue in highly alkaline silica coatings. Down-sides are ecological concerns in regulatory processes and the contribution to AOX-waste water parameters.

Even more active against algae and used in even lower concentration are triazine derivatives. The first one in the film preservative market segment was the 2-Methylthio-4-tert.butylamino-6-cyclopropylamino-1,4,5-triazine (IRGAROL 1071 – Mctt) [II, 20.4.], which was developed to be an antifouling agent for ship paints. Only slightly less active is the very similar, but less costly Terbutryn, having an ethyl-group instead of the Mctt-cyclopropyl. Terbutryn was used long time before as crop protection agent in maize and other cultures. Terbutryn is a little less active than Mctt against a variety of algae species in nutrient broth, but as it is slightly more mobile in coatings this might compensate for the lower activity in certain cases. The upsides of the triazine derivatives are high activity, low leachability, and high stability, even in very alkaline environments like the silicate paints. The main depletion mechanism from a coating seems to be evaporation as the vapor pressure is quite significant. In practice it is frequently observed that algae thriving on triazine containing coatings acquire insensitivity against the active substances. If the triazines are available only in sub-lethal concentration at coating surfaces they are metabolized and even used up as nitrogen source for microorganisms. The triazines have a slightly unbalanced spectrum of activity; some algae need much higher available concentrations than most of the common ones. An interesting development could be the use of combined algicides of the triazine and the dimethyl-urea class, to balance out the spectrum and to complement the different migration behavior in the coating. Neither dimethyl-urea derivatives nor the triazines possess any bactericidal or fungicidal properties, in general; they are only useful to be applied in combined film preservatives.

From the plant protection area there are further herbicides available with significant algicidal activity. Due to the severe regulation requirements and the reluctance of the pesticide companies to apply plant protection agents outside their control further spin-offs are not easily achieved, but it seems to be the only option for innovation on molecular basis in the field.

As could be conducted from the facts described above, no single substance is available to fulfill the requirements of comprehensive coverage of all microbiological threats. The consequence is, that film preservation means combination of different active substances in one formulated product. Combination allows to keep the active substance concentration as low as possible for each component. The negative effect of an authority “over-regulation” can be studied by comparing the film preservatives used in the USA and the rest of the world. While in the USA the first formulated combination preservatives containing Carbendazim, Octylisothiazolinon, and an algicide were not registered with EPA before 1996, this type of combination was preferred in the rest of the world since the early 1980s. From a market survey on German stucco for the finish of EIFS, it can be concluded that the combination of active substances in film preservatives is state-of-the art (Lindner, 2000).

5.14.5.5 Film preservation test methods

As film preservatives have a long-term effect, lasting several years in a coating, there is high interest in accelerated microbiological testing of coating materials. It has to be born in mind, that a coating in long-term service is faced to very different climatic conditions all over the world, and that the material itself is by far not constant over the years. That is why the most important point of a test method for film preservation should be the scope definition. It should be clear-cut, that no single laboratory method could reflect the conditions in practice. Conflicting data may be obtained from accelerated laboratory tests versus long-term exterior exposures, when determining the fungal resistance of organic paint films. Similar imbroglio has

Table 9 Analysis of thick coatings (stucco) for EIFS in Germany (Lindner, 2000)

Coating type - binder:	Polymer dispersion	Silicate	Silicon resin
Total number of coatings investigated:	17	6	6
BCM	13	3	4
OIT	12	2	4
DCOIT	1	–	–
Mctt	6	1	2
Terbutryn	6	1	1
DMCU	3	1	1
(others or none detectable):	1	3	2

resulted from other accelerated testing of paint failure, i.e., salt fog testing for corrosion, adhesion on galvanized steel, and UV light resistance of paints. As film preservatives give a significant contribution to coatings material costs, producers are interested to optimize their use and to predict life-time in service. In most of the cases, zero-defect levels are not really what is targeted, because covering the worst case would mean overdosing for the normal scenario. It is important as well for a reliable producer, to support efficacy claims in front of the customer by experimental data. For the manufacturer and developer of film preservatives the comparative assessment of selected features, like e.g. leachability, efficacy against certain microorganisms, etc., is of utmost importance. For regulatory purposes an efficacy test should support label claims. As a “gold standard” test the statistical evaluation of field data could be considered. Running exterior exposure studies on real objects or on test fences in different climates of the world for at least 2 years gives a valuable set of data to compare different antimicrobial protection. In the laboratory the analytical work to study depletion characteristic of biocides was developed to be an important tool for the researchers.

To design accelerated microbiological laboratory test methods a standard scheme can be summarized from the variety of standard methods and in-house methods applied in the testing laboratories in industry, academic, or institutional testing laboratories, respectively.

- Film preservatives are incorporated homogeneously into the given paint
- A defined film is applied on a given substrate
- After drying the film is exposed to some kind of artificial or natural weathering
- The weathered paint film is challenged by relevant microorganisms under controlled conditions
- After a defined exposure the growth of microbes on the coating is evaluated

This concept is flexible to adapt the relevant microorganisms to be tested, different weathering conditions, different aspects of coating features, but it can not be more than a guideline. Artificial weathering regimes can be adapted e.g. from the wood protection EN 84 or EN 73, or from standards describing the operation of artificial weathering machines.

The International Biodeterioration Research Group (IBRG), originally founded under the auspices of the Organization for Economic Co-operation and Development (OECD) is made up predominantly of scientists from industrial users of biocides, biocide manufacturers, academic institutions, national testing institutes and organizations (www.ibrg.org, 2002). The main objectives of IBRG in the field of test method development and work to investigate basic principles of biodeterioration of coatings cumulated in the development of the standard method BS 3900 G6 for fungicides and BS 3900 G6 draft for algicides. Singapore is the only country in the world asking to pass an algicide standard test SS 345 for coating to be applied on public buildings.

Renovation of surface coatings. Microbially defaced coatings have to be treated carefully while renovated. Of course, it is very helpful to know why the coating failed to keep a clean appearance to avoid the repetition of mistakes by choosing the correctly protected coating material. However, before an infested surface is repainted, the existing biofilm has to be killed and removed. In cases of thick bio-masses adhering the surface, a mechanical treatment, either by brush or a water jet, should remove this layer. Care has to be taken, not to breathe the spores liberated. After that, the surface should be soaked by a masonry microbicidal product. No excess microbicide should leak into the ground. Today, bleach or other oxidizers are commonly used. More user friendly masonry microbicides are based on cationic quaternaries, some of them containing further active ingredients like e.g. octylisothiazolinones, chloroacetamide, formaldehyde releasers, CIT/MIT and other surface active components. Probably, regulatory concerns will decrease the number of available chemicals in highly regulated markets. Under the European BPD, only very few active ingredients will be registered for this market segment 10,- masonry biocides, because the segment is rather limited.

The main purpose on the surface while drying is an eradication of hyphae, already penetrated into the substrate, and superficial left overs from the mechanical cleaning. After complete drying, a penetrating primer can be applied if necessary, then the surface should be finished with a hydrophobic renovating paint containing a carefully chosen film preservative.

Cleaned EIFS – surfaces should be over-painted with silicon paints containing a broad spectrum film preservative.

5.14.6 Antifouling coatings

Virtually all under-water surfaces are exposed to marine organisms causing biological fouling which is only inhibited by the applications of antifouling coatings. It is especially the fouling of under-water surfaces of ships which results in economically most burdensome problems. In the late 1960s it was found that tributyltin (TBT) derivatives, already known as broad spectrum biocides, are also highly effective against a wide range of marine organisms. This led to the worldwide applications of TBT derivatives in antifouling paints.

Table 10 Paint film fungicide test methods (www.oecd.org, 2000, Bagda, E., 2000)

Standard	Title	Comments
ASTM D5590	Test method for determining the resistance of paint films and related coatings to fungal defacement by accelerated four-week agar plate assay	Filter agar nutrient test
ASTM D3273	Resistance to growth of mold on the surface of interior coatings in an environmental chamber	• High variability due to soil. Different substrates will give different results • Ranking can be done only in a single matrix
ASTM D3274	Standard method for evaluating degree of surface disfigurement of paint film by fungal growth or soil and dirt accumulation	• Evaluation of growth on surfaces • Not a performance test
ASTM 3456e1	Standard practice for determining by exterior exposure tests the susceptibility of paint films to microbiological attack	• Ranking is matrix specific and site specific • All factors which affect susceptibility of paint are mentioned
BS3900 G6	Method of test for paints – Assessment of resistance to fungal growth	Designed to predict field performance but is absolutely matrix specific
Federal Test Method 6271.1	Mildew resistance of organic coating materials	Filter paper method, matrix specific
Nordtest 480-84	Assessment of resistance to fungal growth	Like BS3900 G6
JIS 2911	Fungal growth resistance of paint	Filter paper method – mixture of fungi
NF X 41-520	Test of paint, resistance against fungal growth	3 parts: • soil burial test • test on incomplete agar with fungi mixtures • test on complete nutrient-agar with fungi mixtures
SNV 195 121	Materials including paint, resistance to fungal growth	Filter paper test method on agar
VdL-Guideline 06	Determination of the resistance of exterior coatings against fungi	Filter paper test method on agar nutrients – fungi mixture, leaching or artificial weathering

However during the 1970s it was recognized that organotin compounds even at concentrations in the ppb range disturb the biocenosis of surface waters considerably. In the following years it became manifest that the use of antifouling coatings containing organotins are causing severe problems in the marine environment, because of their ecotoxicity. The discussion to ban the use of TBT derivatives in antifouling paints started. In the mid-1980s the first countries forbade the application of antifouling systems that include organotins acting as biocides. In the meantime the Marine Environment Protection Committee (MEPC) of the International Maritime Organisation (IMO), has approved a resolution to phase out and eventually prohibit the use of toxic organotin derivatives in antifouling coatings. IMO set a deadline of 1 January 2003 for a ban. A second deadline of 1 January 2008 also was set for the complete prohibition of antifouling coatings containing organotins.

Due to the environmental concerns over the use of organotin biocides in antifouling coatings, which led to the mentioned resolutions, the search for substitutes the use of which does not result in adverse effects on the marine environment has been opened.

There are available biocides which are highly toxic to target organisms without disturbing the marine environment, as they degrade quickly as soon as they are released to sea water. However, their spectrum of effectiveness is limited. The use of mixtures of such so-called 'booster' biocides, especially with copper, have proved as suitable replacements for organotin compounds in antifouling paints. Research and development with the aim to reconstitute antifouling coatings with long lasting, the environment not negatively influencing efficacy is also demanded.

For further details to the topic see Chapter "3. R&D in Material Protection: New Biocides" which contains also a table listing booster biocides. Their chemical and physical properties, toxicity and antimicrobial effectiveness are described in Part Two-Microbicide Data.

5.14.7 Summary

The hope of mankind, to live in beautiful, multicolored, and clean homes could only become reality for more people, if resources are efficiently used and architectural coatings keep the initial properties as long as possible. Prolonging renovation intervals is possible, if microbial defacement of coatings could be avoided by appropriate

Table 11 Paint film algicide test methods (www.oecd.org, 2000, Bagda, E., 2000)

Standard	Title	Comments
ASTM D5589	Test method for determining the resistance of paint films and related coatings to algal defacement by accelerated four-week agar plate assay	Filter agar nutrient test
ASTM 3456	Standard practice for determining by exterior exposure tests the susceptibility of paint films to microbiological attack	● Ranking is matrix specific and site specific ● All factors which affect susceptibility of paint are mentioned
BS3900 G6 (draft – IBRG)	Method of test for paints – Assessment of resistance to algal growth	Vermiculite-bed test – algae mixture Designed to predict field performance but is absolutely matrix specific
SISIR SS345	SISIR specification for algal resistant emulsion paint for decorative purposes	Trentepohlia – specific, direct inoculation of paint film
ITECH, Lyon	Assessment of resistance to algal growth	“Aquarium-Test” Humid chamber with integrated leaching, algae mixture, non-sterile conditions
VdL-Guideline 07	Determination of the resistance of exterior coatings against algae	Filter paper test method on agar nutrients – algae mixture, leaching or artificial weathering

film preservation. Both, the in-can and the film preservation of coating materials, is a domain of formulated combinations of several active substances and additives. Generally, the preservatives used consist of at least two microbicidal actives to achieve the broad spectrum necessary.

As have to be proven for all applications of biocides, the benefits of surface coating preservatives should out-weight by far the costs and the downsides for health and environment. The long term, close cooperation of coating material producers and the preservative industry made the development of the existing efficient coatings reality. Authorities all over the world have the responsibility not to destroy further progress by “over-regulation”.

Microbicides are the presupposition for modern, environmentally, ready-to-use surface coatings. The ongoing development of paints with always lower organic emissions and better biodegradability is only possible with modern in-can- and film preservatives.

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5.15 Pulp & paper

G. CORBEL

5.15.1 Introduction

A paper mill is manufacturing paper from a source of fibre (wood, market pulp, recycled paper and board) and water. A fiber suspension of around 4% consistency is made in the stock preparation area of the mill and a paper web is prepared on the papermachine, sending the suspension through a slice (the headbox), then on a continuous wire for free dewatering of the stock (white water separation) and in a press section for water drainage, and through a drying section to remove most of the remaining water and get a paper sheet with less than 5% water.

Special surface treatment of the paper can be done on machine using a size press (starch addition) or a coater (coating color contains fillers like carbonate, clay, titanium dioxide and a latex as binder).

The paper reels of several tons are then sent to a converting unit for transformation.

In a typical paper mill:

- 100 to 1500 tons of paper is produced per day;
- A water consumption can vary from 5 to 80 cubic meters of fresh water per ton of paper depending on the system closure;
- Volume of tanks and piping for stock preparation can go from hundred to thousands of cubic meters;
- Volume of the recirculation loop of the papermachine varies from 100 to a few hundred cubic meters;
- Temperature of the recirculation loop of the machine is between 30 and 55°C, similar to that of incubators used for cultivation of micro-organisms;
- The process very often uses starch in addition to cellulose. The nutrient content for certain micro-organisms is therefore high.

Stock preparation systems. All paper mills have a stock preparation system for preparing the paper stock going to the paper machine. Some systems are very elaborate, using automatic proportioning devices, while others are very simple. The age and type of paper machine that the system serves usually influences the type of system.

The stock preparation systems:

- Control the amount and type of stock used at the paper machine.
- Allow various additives to be mixed in with the paper stock.

Paper machine:

The paper machine is the mechanical system used to convert the pulp suspension into a sheet of paper or paperboard. Until recently, the only type of paper machine used was the Fourdrinier design.

The typical method of manufacturing paper involves feeding a mixture of fiber and water onto a travelling wire screen (Fourdrinier wire) so that most of the water drains through the screen and a wet web remains. The web is then subjected to vacuum and pressure (in the presses) to remove more water and receives the final drying by passing through a series of steam-heated cylinders.

Paper stock (or furnish) may consist of a mixture of cellulose, filler (for example clay, titanium dioxide, calcium carbonate), rosin, alum and water brought together in predetermined proportions. The percent of fiber and other furnish materials suspended in the total volume and weight of water is commonly known as “consistency”. Control of furnish consistency in the stock preparation and papermaking area is a prime prerequisite of paper making. The Fourdrinier paper machine was developed for the specific purpose of converting this paper furnish into a finished reel of paper on a continuous basis.

The prepared furnish material for the Fourdrinier paper machine is stored ahead of the machine in the machine chest (stock chest). The furnish is agitated continuously to maintain an even blend of furnish materials. Stock consistency in the machine chest is typically 3 to 4%.

The basic sheet-forming process on a Fourdrinier machine is as follows:

Stock from the machine chest is pumped to a consistency regulator or stuff box from which it passes through a flow-regulating gate (stuff gate) or flow-controlling valve (basis weight valve) and it is delivered to the inlet of the fan pump. In the fan pump, the stock (which is at 3 to 4% consistency entering), is diluted to about 0.2 to 1.0% consistency by water recycled from the wire pit or white water silo. The dilution water is, of course, white water that has drained from the sheet on the wire, collected in the wire tray and returned to the wire pit or silo for dilution of the fresh stock coming to the fan pump.

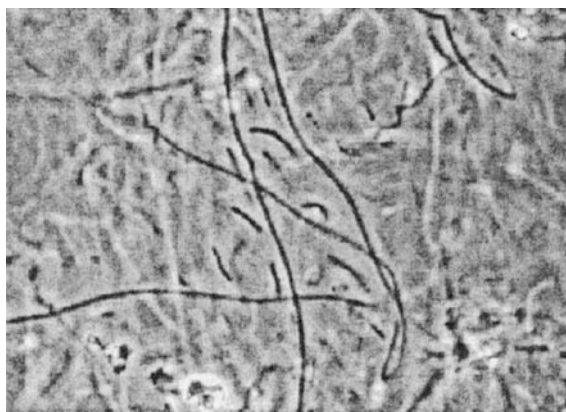


Figure 1 Filamentous bacteria ($\times 400$) in deposit on a paper machine.

The diluted stock may then be pumped by the fan pump through a series of cleaners and into the machine headbox. An inlet distribution header spreads the stock flow evenly over the width of the headbox. The flow is stabilised by the headbox and passes from the headbox through an adjustable orifice plate called the slice. It is discharged onto the moving wire screen (which is usually of plastic construction), dewatered by a series of devices called table rolls, foils and flat boxes; leaves the wire as a formed web of paper. It is further dewatered and dried in the succeeding operation before being wound on the reel at the dry end of the machine.

The variations of mechanical devices from one machine to another are many. However, the final result is the same, formation of a uniform basis weight, continuous web of paper.

As the level of technology improves, higher and higher machine speeds have become possible. Typical machine speeds with Fourdriniers may range from 400 to 3000 fpm (122 to 915 m/min). The advent of twin wire machines (with a wire both above and below the paper web) and vertiformers (with the sheet being formed vertically to the ground rather than horizontally) continues to increase the capacity and capability of the Fourdrinier.

Along with these higher speeds comes the need for chemical applications in the areas of improved retention, better foam and deposit control. Thus, the opportunities for special chemical applications will continue to grow, including biocides to prevent microbiological growth.

Our objectives are to focus on aspects of microbiology that give practical information about microbial problems in the papermaking system. See Fig. 1–3.

Microorganisms can interfere with papermaking by:

- Creating deposits in the papermachine circuits on frames, spots and holes in paper,
- Undergoing anaerobic growth causing smell problems and corrosion,
- Degrading additives polymers, starch, fillers, furnish.

5.15.1.1 Problems due to micro-organisms

Defects in paper sheet. The biofilm or thin slime deposit can further increase in size as it entraps the wood fibers, carbonates, clays and other particles normally used in the papermaking process.

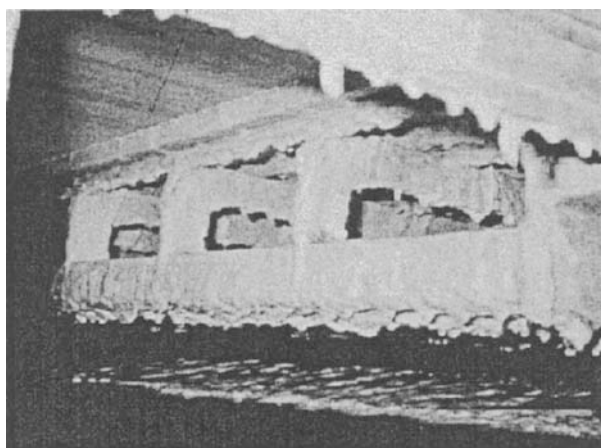


Figure 2 Slime on machine frame of a paper machine.

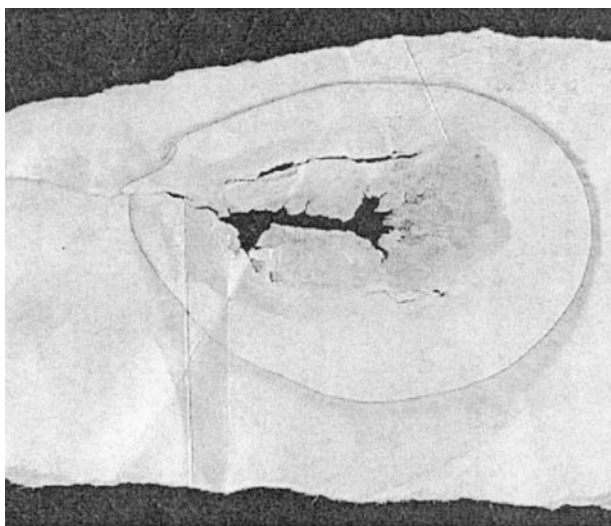


Figure 3 Spot and hole in paper due to slime.

At times, a sticky or polymeric matrix may be present. The possible origins of the sticky materials may include the production of exo-polymeric materials by bacteria, pitch, or even upsets in the chemistry of the papermachines that would result in, for example, gelled alum.

The biofilms in papermachines form massive slime deposits that can be an inch or more in thickness. When these deposits break loose and fall into the paper furnish, they result in end product defects such as holes and spots or even paper sheet breaks. When this occurs, the paper with the defects must be used as broke and re-pulped or downgraded. If the paper containing these holes makes its way to a new high-speed coater, massive problems may result.

If microbial deposition is not controlled not only can it interfere with runnability, but it can also slough from surfaces leading to spots and/or holes in the finished sheet.

Surface deposits and sloughing also serves as an additional source of contamination; therefore, control is critical to the cleanliness of the system.

Operating costs of microbiological growth in paper process. Uncontrolled microbial growth in the papermaking process adversely affects machine runnability and product quality. This results in numerous costs, some of which are not readily obvious to the papermaker. The most easily identified cost is downtime, which can result in losses in profit in excess of 10 000 US\$/hour in a modern fine paper machine. Besides the direct cost of downtime is the expense of paper that must be downgraded or broke. Even more significant are complaints by customers when they discover off-spec paper products.

To summarise:

- Waste of production time,
- Product quality loss,
- Broke or down graded paper,
- Mechanical cleaning,
- Boilouts,
- Stress to wastewater system.

5.15.1.2 *Microorganisms common to papermaking systems*

This section describes the four main groups of microorganisms that colonise papermaking systems and their significance.

- Bacteria: aerobic, anaerobic, filamentous, blue-green algae (cyanobacteria).
- Fungi: yeasts and molds.
- Algae: diatoms, green algae.
- Higher life forms: protozoa, rotifers, nematode worms.

5.15.1.2.1 Bacteria. Bacteria are the most numerous microorganisms in mill systems, with populations typically ranging from 1,000 to 100,000,000 colony forming units (CFU) per ml of stock or additives.

Aerobic bacteria. In a typical, well-agitated paper system many of the bacteria grow aerobically and utilise oxygen for growth. *Pseudomonas* species are common rod-shaped bacteria frequently found in the bulk fluids

and in deposits from paper systems. They use a wide variety of nutrients and grow over a wide temperature range, 41–131°F (5–55°C). Other aerobes commonly found in mill deposits include *Flavobacterium*, *Alcaligenes*, *Zoogloea*, and *Bacillus*, to name a few. Many of these bacteria are slime-formers, producing large amounts of extracellular polysaccharide (EPS) or capsule material when they attach to surfaces or are exposed to adverse conditions.

Facultative bacteria. Facultative bacteria use oxygen to grow (aerobic respiration), but will continue to grow in the absence of oxygen using anaerobic pathways (fermentation). Coliforms are non-sporeforming, rod-shaped, facultative bacteria that are common to papermaking systems. Coliforms, such as *Klebsiella*, *Enterobacter*, and *Serratia*, are normal inhabitants of the intestinal tract of animals, and are found at high levels on plant materials. The temperature of most paper machines is near that of the human body, 99°F (37°C), the ideal range for the growth of coliforms. Coliforms notoriously produce large quantities of exopolysaccharides or slime.

A coliform not common in papermaking systems is *Escherichia coli*. *E. coli* is closely associated with fecal contamination. If *E. coli* is found in the paper system, it indicates that sewer lines are entering process waters, or that faecal waste from birds or other animals contaminates the system.

Anaerobic bacteria. Anaerobic bacteria do not use oxygen and may be harmed by it. Anaerobes thrive in poorly agitated chests, and in areas of deposits in well-aerated chests where oxygen has become depleted. Biofilms are several hundred cells thick, and the microorganisms in the outer layers rapidly use up available oxygen. This results in anaerobic conditions within the biofilm that will favour the growth of strict anaerobes.

When stock or additives remain unagitated for extended periods during shutdowns (>12 h), oxygen is depleted as a result of microbial activity and populations capable of anaerobic growth will be favoured. Anaerobes can cause foul odors in chests (“sour stock”) due to the accumulation of by-products from anaerobic metabolism, including volatile organic and fatty acids (V.F.A.s), poisonous gases (e.g. hydrogen sulphide), and explosive gases (e.g. methane and hydrogen). Gases produced by anaerobic bacteria have resulted in explosions and fatalities.

Two distinct and problematic types of strict anaerobic bacteria are the sulphate-reducing bacteria (SRB), such as *Desulfovibrio*, and the endosporeforming rod-shaped *Clostridium*. *Clostridium* can grow over a wide temperature range, 50–149°F (10–65°C) and can be difficult to control because of the resistant endospores, protecting them from oxygen, heat, desiccation, and chemicals. Explosive levels of hydrogen generated by thermophilic *Clostridia* in a large storage vat caused the death of welders at a mill in Canada in the 1990’s.

SRB have been implicated in localised pitting corrosion, commonly referred to as microbially influenced corrosion (MIC). These and other anaerobes cause biocorrosion due to production of acids and other by-products (e.g. hydrogen sulphide), that cause cathodic depolarisation of metal surfaces. SRB also cause discoloration problems and production of foul odors. They are susceptible to biocides, and can be controlled. Many other types of anaerobes may be present in the mill system, including the common helical-shaped, motile bacteria *Spirillum*. These corkscrew-shaped anaerobes are often seen under the microscope swimming in deposit samples and soured stock.

Maintaining well-agitated systems with low chest levels, and performing thorough wash-ups and boilouts to remove deposits are recommended to avoid anaerobic growth and its associated problems.

Filamentous bacteria. Filamentous bacteria are a group of bacteria that form long chains of cells. These include *Beggiatoa*, *Haliscomenobacter*, *Sphaerotilus*, *Gallionella* and many others. Several species produce a protective sheath structure, a tube-like structure that encloses the cells. The sheath structure may help protect the cells from oxidants and biocides, making these organisms more difficult to control.

Filamentous bacteria usually enter the paper machine system with the incoming fresh water. They can be a significant problem in mills operating at neutral to alkaline pH, which is optimal for their growth. Filamentous deposits may show up as long white, yellow, pink or orange gelatinous tufts or streamers. These can plug shower nozzles and felts and generate thick deposits due to the strong slime matrix formed. These biofilms can serve as a nucleus for the deposition of other suspended solids.

Filamentous bacteria are typically detected microscopically since they do not grow readily using standard plate culture methods. Under the microscope you can often identify iron-depositing bacteria by the orange iron oxides deposited within or around their sheath (e.g. *Gallionella*). Sulphur-oxidising bacteria are very long and large in diameter and can be readily distinguished under a phase contrast microscope by numerous bright, refractive sulphur granules deposited inside the cells.

Bacterial protective mechanisms: slime formation [I,5.I].* Most environmental and industrial systems exhibit wide variations in conditions (pH, temperature, oxygen content, nutrients, toxic chemicals, etc). Often these

*Part I, ‘5.1. Efficacy of Biocides against Biofilms’

variations are outside of the optimal conditions for biological growth. When conditions become less than favourable for microbial growth, certain bacteria have developed protective mechanisms to ensure their survival.

The protective mechanisms employed vary from common to highly specialised. These include:

- Excretion of exopolysaccharide (slime),
- Capsule formation,
- Bacterial endospore formation,

Slime. The excretion of polysaccharide (slime) can be thought of as a defence mechanism. Unlike a capsule (see below), the polysaccharide is loosely attached to individual cells. This slime layer aids in attachment to surfaces, helps prevent dehydration, slows diffusion of harmful substances, traps debris and nutrients, and can even serve as a source of nutrition when required. The production of slime results in a biofilm or biofouling. Biofilms represent the predominant manner in which microorganisms live in nature.

Capsules. They are a protective structure, which may be produced by some bacteria. Capsules consist of polysaccharide, polypeptide or both and are firmly attached to the cell wall. It is thought that the presence of a capsule protects the cell from phagocytosis (being eaten) although the mechanism is not known. The capsule can prevent from dehydration and trap nutrients. Also, the sticky nature of the capsule aids in the attachment of the bacteria to surfaces. The significance of capsules in papermaking systems is not known.

Endospores. They are unique to bacteria and are limited to only a few genera. Spores are survival structures that are highly resistant to heat, desiccation and exposure to toxic chemicals. They are formed within the bacterial cell and store the genetic material and other cellular materials that are necessary to resume metabolism once environmental conditions become favourable. Spores are much smaller than the original bacterial cell. Only one spore is formed per bacterium. Endospores can remain dormant for thousands of years and withstand boiling temperatures for several hours. They can survive the dryer section of a paper machine and be detected in the finished sheet. It is suspected that spores can survive jet-cooking conditions in many cases. Spores are particularly problematic in Dairyman's Grade or aseptic packaging grade paper.

5.15.1.2.2 Fungi. Fungi include molds and yeasts. Most fungi prefer acidic environments where they tend to outcompete bacteria, but they can also grow at alkaline pH when bacterial growth is controlled. Fungi are commonly found in mill furnishes run at acid pH, as well as additives: clays, alum, dyes, and uncooked starch tanks. On wet machine surfaces, such as misted areas around foils and frame, fungi form tough, leathery mats. These are difficult to remove with a wash-up hose. Because fungi require much lower water levels than bacteria for growth, they grow very well on wet-lap and even in dry-lap pulp or paper stored under conditions of high humidity.

Moulds reproduce through hyphal fragments or spores that germinate under optimal conditions. Fungal spores are much less resistant than bacterial spores to drying, radiation, heat and chemical agents. However, fungal spores can survive some machine dryers and later germinate and grow in the finished sheet, under humid conditions.

In general fungi do not reproduce as rapidly as bacteria.

Fungi are a primary cause of microbial degradation of wood, and cause cellulose loss and reduced brightness in wood-chip storage piles.

Yeast. Yeast are troublesome because they thrive in the white water and form slimy, tenacious deposits on submerged surfaces. Yeast will grow under anaerobic conditions and produce carbon dioxide and alcohol as by-products of yeast fermentation. Yeasts can also lead to problems when they grow in starch slurries and cause fermentation.

5.15.1.2.3 Algae. Algae are found in areas where moisture and light are present and freshwater treatment is inadequate. Algae seldom cause deposit problems, but can occasionally cause a paper defect, such as a green spot in the sheet. Problems related to algae growth typically occur in well-lit, misted areas such as stairs and other surfaces with high foot-traffic, where high densities of algae form a slimy, slippery green mat creating a safety hazard.

The presence of algae in the system indicates inadequate freshwater treatment. If algae have entered the paper machine system, it is likely that slime-forming bacteria are also present. Diatoms are a group of algae that are surrounded by a silica wall that can remain intact even after death. Living diatoms are easy to spot under the microscope due to the presence of green chloroplasts, while nonviable diatoms appear like glass skeletons. This is useful as an indicator of the efficacy of the freshwater treatment program. The presence of intact chloroplasts is an indication of inadequate freshwater treatment.

5.15.1.2.4 Microfauna. Microfauna, including protozoa, metazoa, and worms (e.g., nematodes), are small, complex animals that feed on bacteria and frequently inhabit deposits. These organisms are easy to see under the microscope because they are much larger than bacteria and are usually very active.

These organisms are not the root cause of deposits, but their presence indicates inadequate treatment of incoming fresh water. If these organisms are present, the fresh water will likely serve as a source of entry for slime-forming bacteria as well. Mills with good treatment programs may occasionally find microfauna in misted areas that do not come into contact with biocides.

The presence of worms and rotifers also indicates that the deposits have been stable for an extended period of time, suggesting poor housekeeping and inadequate wash-ups or boilouts.

5.15.1.3 Biological deposition on paper machines

A paper machine re-circulates hundred to thousands of cubic meters of water at a pH from 4 to 8 and a temperature from 30 to 60°C. It contains huge amount of nutrient such as starch and can be contaminated by air, water (3 to 60 m³/ton of paper) or furnish (recycled paper) and additives. In many ways, it can be considered as an incubator with 10² to 10⁸ bacteria/ml. See Fig. 4-5.

5.15.1.3.1 How do micro-organisms grow on machines? Biological contamination will have two different aspects of development in paper process:

- *Sessile* = attached to the surfaces like pipes, headbox, wet end parts: filamentous bacteria and rod shape bacillus are typical of such contamination in alkaline process.
 - Biofouling sources,
 - Survival advantage,
 - Permits growth of diverse types,
 - Difficult to control,
 - Provides seed for planktonic state and spoilage.
- *Planktonic* = free-swimming in the pipes and in the tanks

Planktonic bacteria are measured with plate counts e.g. of a head box sample.

Sessile bacteria are measured either by analysing deposits taken from the machine, or by using a fouling monitor, or following the rate and nature of deposition on a reference surface in the machine using either a microscope to see individual sessile cells or enumerating some of the microbes present by plating the actual deposit.

The sessile bio-film in a paper machine contains many different types of microbes. Many can not be recovered using conventional plating media and specialised techniques may be needed.

Just because bacteria are present in the fluids does not mean that they will form a deposit.

Counts of the planktonic population may give the potential for fouling.

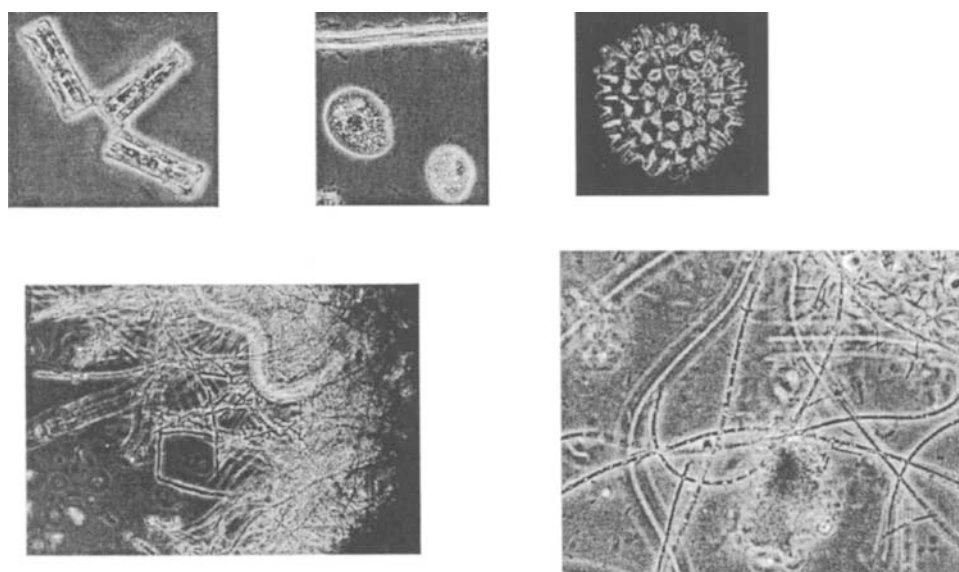


Figure 4 *Typically enter via fresh water.

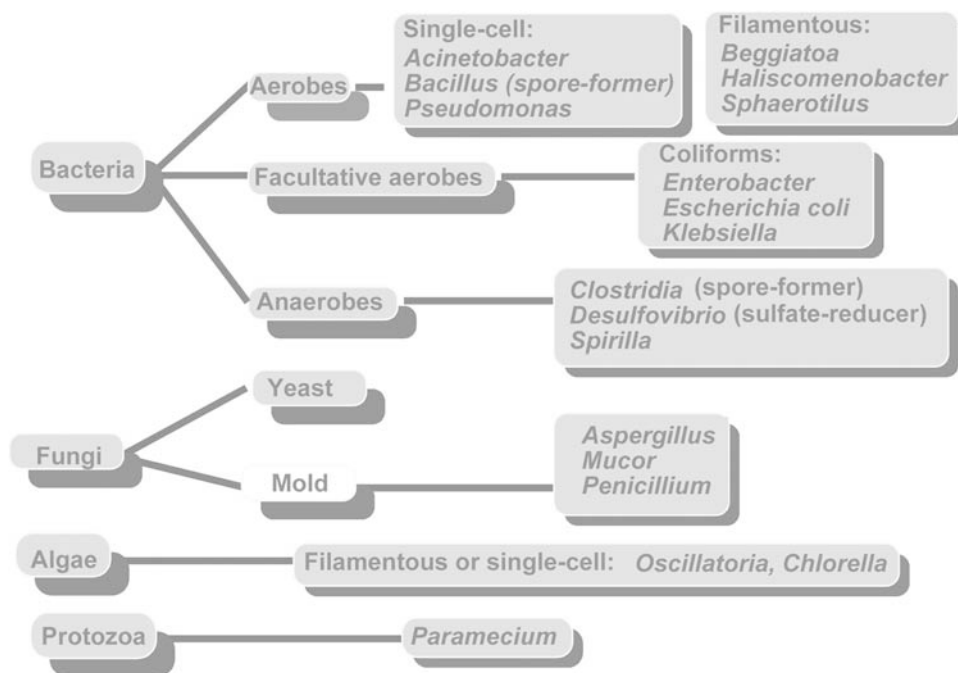


Figure 5 Microorganisms growing in paper machine system.

One machine can operate well with high counts in the 10's of millions and have no deposition. Another machine can have less than 10 000 cfu/ml in a sample and be heavily fouled.

Filamentous bacteria are rarely measured in planktonic counts and grow poorly on Petrifilms. Many will not grow on artificial media at all. Filamentous bacteria form major deposit problems (sessile population) on neutral to alkaline machines.

5.15.1.3.2 Model of biofilm growth. Although some people use the paper machine as a fouling monitor for sessile growth, it is not recommended!

Research has shown that while it may take 1 ppm of residual chlorine to kill planktonic population but it may take 1000 ppm to kill a sessile population.

A sessile organism is protected from biocides by polysaccharide slime and changes in cell metabolism.

Deposit control polymers or cationic biocides like quaternary ammonium may be more effective in preventing planktonic population from attaching than some biocides that may give better planktonic kill.

However keep in mind that you need to control planktonic population to keep fibers and additives free from spoiling.

5.15.1.3.3 Origin of deposits. Most deposits are composed of both chemical and biological elements. Do not always assume that the cause of the deposit is microbiological. Other materials may cause the initial deposit, act as source of nutrients, and lead to the fixation of microbes on surfaces, that can be observed thanks to an optical microscope:

- Filler, wood fiber fibrils: Bacteria are present at low but significant levels. If there are 1–2 bacteria per field, you will have about 1 000 000 colony forming units per ml (cfu/ml). This is a general rule and should be used as an indication only. It can not be used in place of plate counts.

The information in this microscopic field tells us that we need to do a better job of washing up of splashed stock. This deposit can turn into a microbial deposit problem if it sits too long without being removed. Retention critical to deposit control!

- Pitch can increase microbial deposit problems. Sticky & Hydrophobic.
- Presence of nematode worms and protozoa demonstrates a failure of the fresh water treatment.
- Analyse of the deposit with FTIR would show information about a mixture of chemicals: fillers, lignin, amines.
- × 200 magnification microscopy can:
 - Give evidence of fibers and additives,
 - Indicate the underlying cause: bacteria as threads, filamentous bacteria.
 These filaments bind deposits together making them difficult to remove.

5.15.1.3.4 Consequences of microbiological growth on paper process.

- Fiber strength loss.

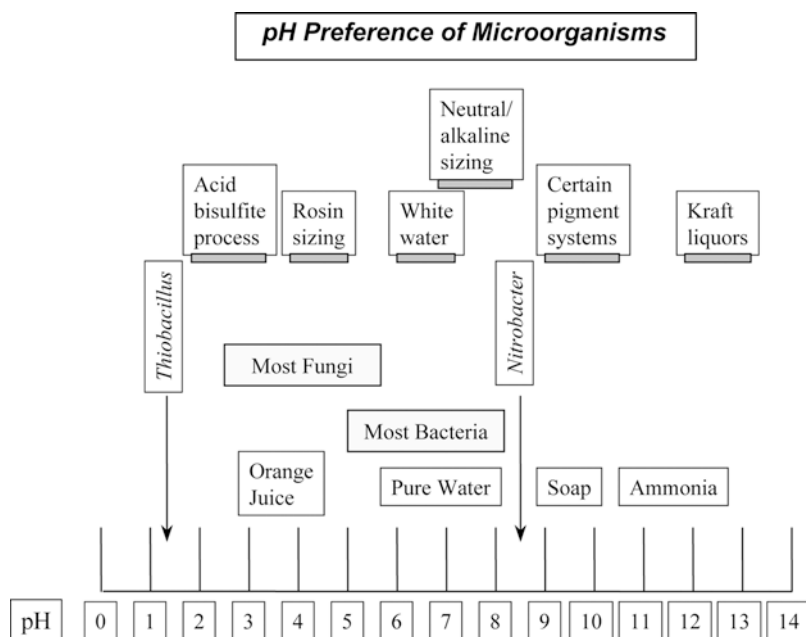


Figure 6 Process pH and bioactivity in papermaking.

Spoilage.

- Interference with pulp & coating brightness,
- Reduction in strength in fibers,
- Malodorous acids and even headbox pH swings,
- Toxic and explosive gases: hydrogen, hydrogen sulfide,
- Waste of money for the papermaker,
- Foul smelling paper,
- Reduction in pH,
- Reduction in ORP (Oxide Reduction Potential).

5.15.1.4 Goal of microbial control programs

- Keep the machine free of deposits using biocides or deposit control polymers.
- Prevent additives and pulp from souring or spoiling.
- Keep circuits and tanks aerobic to avoid souring and corrosion.
- Prevent the paper and the mill from bad smells.

5.15.2 Control of microbiological growth in the paper industry (See Fig. 6–14)

5.15.2.1 Non oxidising biocides in paper making

(Introduction to the Microbial Control Manual, ONDEO NALCO P&P LIBRARY, Laura Rice/Linda Robertson dated February 2001).

	Acid	Alkaline
Typical	Fungi	Filamentous bacteria
Predominant Organisms	Single cell bacteria	Single cell bacteria
Typical Minor Organisms	Filamentous bacteria Protozoa Algae	Fungi Protozoa Algae
Biocide need	1 X	2 – 4 X

Figure 7 Alkaline vs acid: effect of pH on microbiological growth.

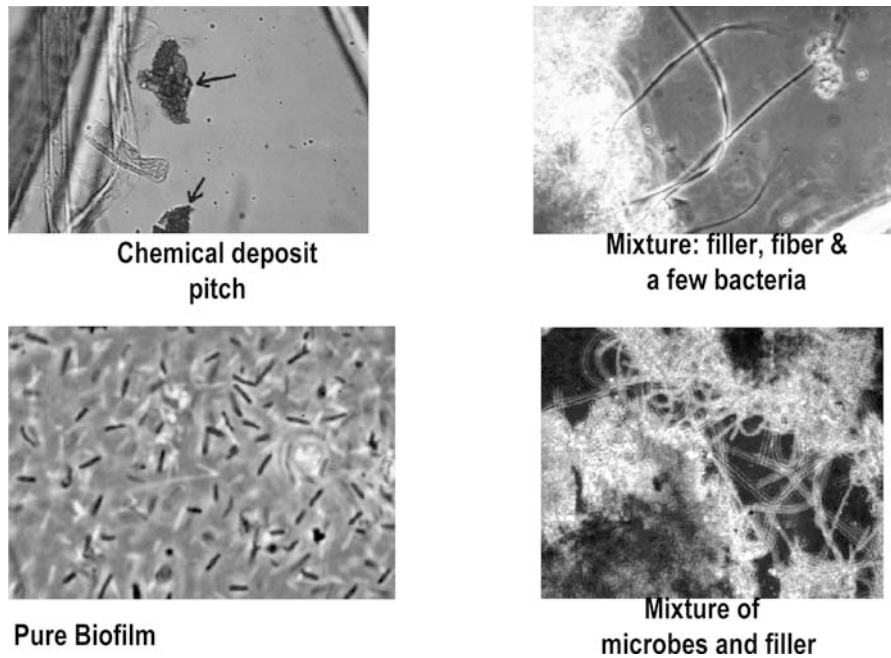


Figure 8 Deposits on paper machines.

Non oxidising biocides are widely used for microbiological control in papermaking:
The main applications areas are:

- Short circuit of the machines to keep clean the headbox and the formation area that will avoid paper contamination, defects in the process and breaks on the machines.
- Treatment of additives like starch, polymers, coating color, fillers to avoid future contamination of paper circuits and keep the rheology and other properties of these additives in specification.
- Fresh water treatment to prevent machine and additives contamination through showers in wet end and press section or dilution of stock.

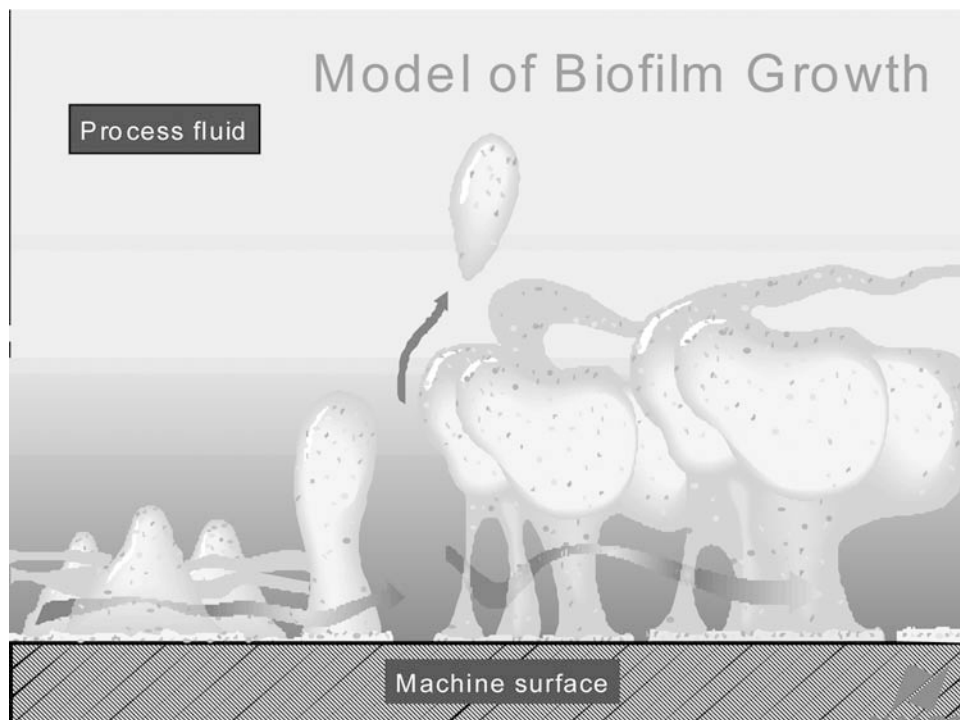


Figure 9 Biofilm model.

FUNGUS RESISTANCE TEST ACCORDING TO TAPPI T 487 CM 93

Test conditions : T : 28° C $\pm$ 1° C R.H. : 90 $\pm$ 5 %

Test started on : December the 7th, 2001

Number of paper samples tested for each side : 3

EXAMINATION OF THE SAMPLES AFTER 14 DAYS

FUNGUS	PAPER REFERENCE	Untreated paper (Nalco blank)		Masterchrome WS 80 without SURFASEPT 74842		Masterchrome WS 80 with SURFASEPT 74842	
	Side of the paper	T	B	T	B	T	B
ASPERGILLUS NIGER	NO GROWTH					3	3
	SLIGHT GROWTH						
	HEAVY GROWTH	3	3	3	3		
ASPERGILLUS TERREUS	NO GROWTH					3	3
	SLIGHT GROWTH						
	HEAVY GROWTH	3	3	3	3		
CHAETOMIUM GLOBOSUM	NO GROWTH					3	3
	SLIGHT GROWTH						
	HEAVY GROWTH	3	3	3	3		

COMMENTS : Two pieces of paper (one « T » with *Aspergillus terreus* and one « B » with *Chaetomium globosum*) show contamination due to *Alternaria*.

Figure 10 Example of Tappi test carried out on paper samples to check the mold proofing treatment.

5.15.2.1.1 Treatment of short circuit of the machine. This is a key application in paper making: 100 to 300 cubic meters of water are recirculating very fast in the wet end part to form the paper web. The diluted stock enters the headbox between 0.5 and 1% consistency to form the paper web that will drain on a wire to remove free water make a paper sheet with a dry content around 20 to 25%. This sheet will then be pressed between press rolls and felts to remove the water bound to the fibers and reach around 50% dry content.

Now the water cannot be removed any more by mechanical means, and drying using big drums heated by steam will allow to reach 95% dry content.

In this process the water after draining the web is recirculated to mix with fibers again and then re-injected in the headbox. This water is highly loaded in fines, colloidal stuff from wood or recycled fibers and starch. The temperature is typically between 30 and 55°C.

The machine can be considered as an incubator which means that slime in the form of stringers or biofilm can form readily and disturb the process. To prevent such bio-deposits to develop, non-oxidising biocides are dosed in the recycled water (called white water).

The dosage is most of the time discontinuous by shock injection.

The choice of the biocide and the dosing regime are dependent upon several parameters such as :

- **pH of the machine:** Typically MBT [II, 20.9.1]* (methylenebisthiocyanate) has been widely used when the machines have an acid pH between 4.5 and 6. Now most of the machines, at least in Europe, are running with neutral process and pH between 7.0 and 8.5. This has led to different slime problems with far less fungi and far

*see Part II – Microbiocide Data

more deposits with bacteria in zooglea masses filming the surfaces and carbonate. This shift to higher pH with different deposits has led to alternative biocides being used for this application: a combination of a fast killer such as DBNPA (dibromo-nitropropionamide) [II, 17.5.] in association with a more persistent biocide (e.g. benzisothiazolinone) [II, 15.6.].

- **Redox potential (ORP):** If there is a reductive environment (i.e., ORP < 100mV), as with a sulphite pulp, a combination program of glutaraldehyde [II, 2.5.] and carbamate [II, 11.] is very effective. The glutaraldehyde works as a quick killer at the alkaline pH, and the carbamate is very persistent, especially when slugged in thick stock. Quaternary ammonium compounds [II, 18.1.] are also very effective in reducing conditions but should be carefully fed in the paper machine system because of their cationic nature that could upset some of the wet end chemistry. If there is an oxidative environment (ORP > 150mV), the combination of DBNPA/Isithiazolinone [II, 15.3.] is effective as long as there is not a high level of bacteria creating reducing conditions and deactivating the biocides.
- **Temperature:** Low temperature (below 30°C) or warm machines (above 50°C that are often observed with TMP and also with DIP treated with hot dispersion) will reduce biological activity and make the machines less favourable for microbial growth. The use of non-oxidising biocides can then be reduced and the treatment mainly based on the usage of polymers/surfactants blend to inhibit the growth of micro-organisms or remove the biofilm.
- **Water consumption:** This is an important parameter because it will determine the contact time on the machine between the dosed biocides and bacteria. Some machines with open system are using up to 60 m³ per ton of paper. Very fast acting biocides like DBNPA will help to keep microbial growth under control. On the contrary, low acting biocides such as Isothiazolinones can be ineffective treating mainly the effluents! In very closed system such as machines manufacturing corrugating board without effluent and with re-injection on the machine of rejected pulp and sewer water, anaerobic conditions can easily occur. In this case biocides such as glutaraldehyde, THPS [II, 3.6.] and sulfones [II, 17.] that are effective under reductive conditions can be very effective in controlling slime deposition.
- **Paper process:** Odours in paper, paper quality like brightness can interfere with the biocide selection. Carbamates can react with metal and reduce the brightness of the paper.
- **Legislation/agreement:** Depending on the usage of the final paper, some agreement or registration will limit the choice of non oxidising biocides: food packaging or hot filtration, cigarette paper have a limited number of potential biocides that can be used (see BgVV, FDA, EPA approvals).
- **Conclusion:** Depending on the physical and chemical parameters of a paper machine, the selection of biocides is quite limited. As a rule of thumb:
 - To inhibit microbes, use a preservative,
 - To reduce the population, use a killer,
 - Ensure the inhibition of biofilm formation.

SYSTEM pH		
4,0 - 6,8	6,5 - 7,0	7,0 - 9,0
All biocides acceptable DGH only > 5,5	MBT quickly breaks down	MBT less effective
Glut & Glut / Quat act as preservative	Glut & Glut / Quat act as both preservative & killers	Glut & Glut / Quat become rapid killers

Figure 11 Non oxidising biocides and pH.

Preservatives	Killers
Thione [II, 3.3.25.]	Carbamate (high dose)
Carbamate [II, 11]	Glutaraldehyde (alkaline pH)
MBT [II, 20.9.1.]	Glut/quat blend
Glutaraldehyde [II, 2.5.]	Sulfone/quat blend
Adamantane [e.g. II, 3.3.2]	Quaternary ammonium compounds
Dodecylguanidine hydrochloride	DBNPA [II, 17.5.]
Glut/Quat blend	Dodecylguanidine hydrochloride (DGH)
Bromnitropropanediol [II, 17.14.]	
Sulfone [II, 17.]	
Isothiazolinone [II, 15.]	
Quat. Ammonium compounds [II, 18.1.]	

Figure 12 Preservatives vs. killers.

5.15.2.1.2 *Control of bioactivity in additives.* The preservation of additives for papermaking is essential for several reasons:

- Prevent the degradation of the additives: anaerobic bacterial growth can very quickly modify the properties of any additive. Contamination of additive will make it unsuitable for usage;
- pH reduction and thickening can occur rapidly in carbonate filler;
- Starch can be degraded in sugars with loss of effectiveness and spot in the paper;
- Coating color can be heavily affected by microbial activity with a drop in pH and more problematic, changes in viscosity, which means problems in coating;
- Avoid microbial contamination of the paper machine, feeding pipes and filters. Retention-aid polymers are introduced after the cleaners at the last step before the headbox. These polymers can be highly contaminated with fungi leading to the development of dark black slime in the preparation tanks and in the pipes.

The selection of a biocide for additives preservation is carried out according to the following parameters:

- **Shelf life of an additive:** Preservation of carbonate dispersions [I, 5.9.] for transportation from the manufacturing site to the paper machine by road or train can take several weeks. Filler preservation with OPP (orthophenylphenolate) [II, 7.4.1a] or formaldehyde release agent (i.e. dihydroxy-dioxahexane [II, 3.1.4b.], hexahydro-triethyl triazine [II, 3.3.19.]) can be very effective and not affected by ORP reduction in a totally closed tank. But the same filler, when stored in the mill in a container with very fast circulation (and with a storage time from a few hours to a few days) can be treated with Isothiazolinones and bromonitropropanediol [II, 17.14.] through one or a few injections per day.
- **Nature of the contamination:** Polymer dispersions [I, 5.7.] are mainly contaminated with fungi. The fungicide property of the biocide is essential and MBT or Isothiazolinones are typically used for this application.
- **Compatibility with the additives:** Simple parameters such as pH, electric charge, redox, water solubility are critical for successful additives preservation.
- It is recommended to carry out miscibility tests to check that no separation (flotation, sedimentation, coagulation) will occur due to the preservation treatment. This is very important for coating slurries preservation where any rheological change in these complex formulations can destabilise the coating. These tests can include enduction ("draw down test"), brightness test, printability tests.
- A good example of incompatibility is the stabilisation of Isothiazolinone with copper nitrate that is anionic. Destabilisation by cationic molecules (cationic polymers or starches) are reported.

Dosage level: The dosage level for preservation is anything between 100 and 500 ppm of commercial product, dosed from one to several times per day depending on the operating conditions. A treatment for each batch can also be required. All these parameters have to be adjusted according to the evolution of pH, ORP and contamination with time. For starch and coating color, a level of 10^4 cfu/ml is considered as a maximum for total contamination. For fillers, the limit is 10^6 cfu/ml.

5.15.2.1.3 *Fresh water treatment.* Most of the paper machines use fresh water treated with an oxidant, generally an halogen like chlorine or bromine via hypochlorite, sodium bromide, BCDMH [II, 21.2.] but sometimes this is not possible either because the water is used for the boilers or potable water or corrosion is a problem. This leads to a specific treatment being added to the water feeding of the machine, often at the wet end showers at the wire or at the felt high pressure shower, at a dosage level of 5 to 50 ppm, depending if it is continuous or slug dosing.

Then a typical treatment is the dosage of a quaternary biocide or a quaternary/glutaraldehyde blend in the water. Addition of biocide to the water storage tank before the shower is much more cost effective and will allow

discontinuous treatment. Because such treatments are often designed to prevent the development of filamentous bacteria around the machine close to the spray bars, biocide efficiency cannot be controlled by the use of plating or Petrifilm. The best criterion for success is the elimination of slime due to filamentous bacteria.

DBNPA, MBT and isothiazolinones have also been used for this application.

5.15.2.2 *Oxidising biocides [II, 21.] in paper making*

(ONDEO NALCO P&P Rep Conference, Linda Robertson/Judy Lazonby dated February 1998).

Oxidants can act as quick-killing biocides with a broad spectrum of activity. With high enough concentrations and long enough contact times, oxidants will kill all types of bacteria including filamentous bacteria, spore formers and anaerobes. Oxidants, in general, are usually less expensive than non oxidising biocides on paper machines.

Oxidants also provide additional benefits such as enhancing the performance of other biocides. Biocides like isothiazolinone, Bronopol, and DBNPA work much more effectively in an oxidising environment than in a reducing environment. Oxidants can also alleviate anaerobic conditions and decrease the problems caused by anaerobic bacteria such as odors.

Oxidants such as chlorine, chlorine dioxide, and peroxides are also used as bleaching chemicals and are very familiar to papermakers.

Even though oxidants can be very effective and are relatively inexpensive biocides, they can also cause problems for the papermaker. Oxidants can corrode the metals used in the paper machine and destroy felts used in the press section. Oxidants can also react with and destroy other chemicals used in papermaking including dyes, wet strength, and even other biocides.

One advantage in using alternate oxidants such as Peracetic Acid (PAA) and ammonium bromide is that these oxidants are generally less detrimental to equipment and other chemicals used in papermaking.

The main oxidants used for biological control in papermaking are the following:

- sodium hypochlorite (bleach), [II, 21.2.2]
- sodium hypobromite, [II, 21.2.3]
- BCDMH, [II, 21.2.11]
- chlorine dioxide, [II, 21.2.4]
- peracetic acid, [II, 21.1.3]
- chloramine, [II, 21.2.5]

The main application has been the fresh water feeding of the machines but more and more machines are using oxidants for the treatment of process water in the recirculation loop (short circuit) and recovered water (long circuit).

5.15.2.2.1 Chlorine and bleach. Both chlorine gas (Cl_2) and sodium hypochlorite (NaOCl)(bleach) can be used to generate hypochlorous acid (HOCl), a strong oxidiser and good biocide. Chlorine gas dissolves very quickly in water. At pH 4 and higher, the reaction of Cl_2 is complete, with less than 1% Cl_2 remaining and 99.72% as HOCl . HOCl is also readily formed when NaOCl (bleach – a commodity chemical) is added to water. HOCl dissociates to H^+ and OCl^- as pH increases. At pH 7.5, the concentrations of HOCl and OCl^- are approximately equal. This is important because OCl^- is a much less effective biocide than HOCl . However, as HOCl is consumed the equilibrium reaction may shift from OCl^- to HOCl (reservoir effect).

Chlorine gas and sodium hypochlorite are used in fresh water and process water (white water) applications. Some mills are also using hypochlorite for thickstock applications. Because bleach is a relatively cheap commodity chemical, many mills are increasing application of this oxidant to reduce biocide costs. However, bleach can be very aggressive towards papermaking equipment and chemicals.

5.15.2.2.2 Hypobromous acid. HOBr (hypobromous acid) is produced by reacting NaBr (available as a Nalco product) with HOCl (either from bleach or chlorine gas). Therefore, a dual feeding system is required. Chlorine dioxide will not activate NaBr .

It is important to understand the blending ratio of HOCl and NaBr for laboratory testing and field application. If you are doing a lab experiment and only want to generate HOBr , then mix either 0.55 g of NaBr or 1 ml of NaBr solution (~40%) with 5 ml of Chlorox (5.25% sodium hypochlorite) and dilute to 100 ml with distilled water. At this ratio, only HOBr will be produced. In mill applications, it is recommended that a higher molar ratio of $\text{HOCl}:\text{NaBr}$ be used (e.g., 4:1). This ensures that all of the NaBr is converted to HOBr and is not wasted.

In general, HOCl and HOBr have about the same germicidal efficiency. Since HOBr dissociates at a higher pH than HOCl , HOBr is often used in place of HOCl in higher pH systems.

In the presence of nitrogen-containing compounds, HOCl and HOBr form chloramines and bromamines, respectively. Bromamines are more biocidal than chloramines, thus, another potential benefit of using hypobromous acid but one disadvantage of HOBr is that it is very susceptible to oxidant demand.

5.15.2.2.3 Chlorine dioxide. Chlorine dioxide (ClO_2) dissolves in water, it does not hydrolyse. Therefore, HOCl is not formed but may be present from the generation process. ClO_2 can easily off-gas with aeration and increases in temperature. At normal process/fresh water pH, ClO_2 is reduced to chlorite (ClO_2^-) when it reacts.

ClO_2 is not stable to shipping or storage and therefore must be generated on-site. A variety of commercial generators are available for producing ClO_2 . Another source of ClO_2 is pulp mills. Because of the concern of generating chlorinated organic compounds with HOCl, ClO_2 has become a common bleaching agent for the pulp plant. ClO_2 from the pulp mill can be a cheap source of oxidant for the paper mill.

ClO_2 is usually as effective, if not more, as chlorine. Advantages of using ClO_2 include its lack of reactivity with nitrogen compounds as well as its ability to be effective over a wide pH range.

ClO_2 is not recommended for freshwater treatment. This is based on field experience, and probable causes include lack of persistence (flashing off?) and poor filamentous control. However, it is commonly used for process water treatment especially if the pulp mill is supplying it cheap.

5.15.2.2.4 Peracetic acid. Peracetic acid (CH_3COOOH) as formulation also contains hydrogen peroxide, and a special HAZOP pumping system has to be supplied to ensure safe handling. PAA is not affected by catalase but will be neutralised by protein rich in S-H and S-S bonds (casein).

PAA is very synergistic with biocides such as isothiazolinones, and other oxidant biocides.

PAA is used for process water treatment. Compared to halogens such as hypochlorite and bromination, it has several advantages:

- compatible with dyes,
- less corrosive, does not damage felts,
- does not form halogenated organic compounds (AOX),
- may be more persistent.

PAA is used for process water applications and is not recommended for freshwater treatment.

A new 15% PAA formulation has been developed and is currently being implemented into most customers who should make PAA programs more cost effective.

5.15.2.2.5 Stabilised hypobromous acid. It consists of bromine stabilised with sulfamate. When the bromine is released from the stabiliser, HOBr or OBr^- are formed and serve as the biocidal actives.

The product has been used successfully for some fresh water applications. However, in most cases, hypochlorite or hypobromous acid are better choices. At the time this presentation was created, four sold accounts have been obtained for process water application, and a number of trials continue. Based on laboratory and field data, advantages of stabilised hypobromous acid include good persistence, enhanced fouling control, and less aggressiveness towards some papermaking equipment and chemistries.

5.15.2.2.6 BCDMH. BCDMH is similar to products that use an organic molecule to stabilise bromine and chlorine. When the bromine and chlorine are released, they form HOCl, OCl^- , HOBr, and OBr^- . Easybrom is a solid product and is sold as granules, briquettes or powder. A specialised feeder has been developed for the application of this product as powder and slurry in papermaking and is currently being used in paper mill process water.

Based on laboratory studies and some field applications, it has been found to be very effective against filamentous bacteria, work well in some high oxidant demand systems (e.g., recycle board mills), and is generally less aggressive to papermaking chemistries and equipment than non-stabilised halogens.

5.15.2.2.7 Chloramine. By mixing sodium hypochlorite with ammonium bromide, chloramines can be. Chloramines are believed to be nearly as biocidal as hypobromous acid or hypobromite. This product has recently been introduced in. At this time little is known about the product. It requires a somewhat complex feeding system because caustic soda is also added so that the pH can be closely controlled. Furthermore, corrosion problems could be expected on non stainless steel equipment, including in the vapour phase (press and drying sections).

5.15.2.2.8 Key parameters for oxidant treatment.

Oxidant demand. It is the quantity of oxidant that is reduced or converted to inert or less active forms by substances in the water. The level of demand will have a dramatic effect on oxidant performance. Demand may come from organic compounds (e.g., bacterial proteins) or inorganic compounds (e.g., sulphite or amines). Demand is determined by measuring the difference between the amount of oxidant applied and the free oxidant remaining after a given contact time.

Note: Sometimes it is thought that oxidant demand must be met before bacterial kill can occur. This is not true. Demand must be met for a free oxidant residual to exist. Since bacterial components can be responsible for some, if not most, of the oxidant demand, kill can occur as the demand is being met.

Figure 14: Relative effectiveness of bromination and sodium hypochlorite in white water from a mill that uses chlorine dioxide bleached pulp. Note: the performance of bromination and hypochlorite is similar on an active (Cl_2) basis and only 2 ppm are needed for significant kill.

In the second example, white water from a recycle board mill with a high degree of closure was used (very high demand). Even at concentrations as high as 40 ppm actives (Cl_2), the oxidants had little effect. Because of high oxidant demand, oxidants are usually not used in recycle board mill process water treatment.

Figure 13: As mentioned earlier, HOBr has a higher dissociation pH than HOCl. This means that in the pH 7–9 range HOCl dissociation will be more favourable than HOBr dissociation. This is why HOBr is often used at higher pH values. However, it *does not* mean that HOBr will necessarily be more effective at higher pH values. As shown in the previous slides, oxidant demand can have a dramatic effect on oxidant performance outweighing the effect of pH. Furthermore, just because HOCl ionises to OCl^- , it does not mean that it is not available. When HOCl is consumed, to maintain an equilibrium, more HOCl is generated from the reaction of H^+ with OCl^- (reservoir effect).

Contact time. A white water sample was dosed with various concentrations of sodium hypochlorite on an actives basis (8–12.5 ppm Cl_2). Note: as contact time decreases, higher concentrations of oxidant are needed to achieve the same level of kill.

Temperature. As temperature increases, the volatility of oxidants may increase (e.g., chlorine dioxide) resulting in a loss of oxidant.

Oxidants like sodium hypochlorite may degrade rather quickly depending on storage conditions (e.g., length of time, exposure to sunlight, etc). This can result in a significant loss of residual over time.

Other chemicals. Oxidants are very non-specific in their reactivity. Therefore, the presence of chemicals susceptible to oxidation can affect oxidant performance. For example, many oxidants are known to react with dyes which results in a loss of oxidant and increased dye usage. Some oxidants can react with non-oxidising biocides. Some oxidants can even react with each other (e.g., hypochlorite and hydrogen peroxide). Interferences with OBA (optical brighteners), starch, sizing, unbleached groundwood have been reported.

Process water. In process water, oxidants are applied to oxidise the system and provide kill. They are usually applied to the white water or thickstock. However, thickstock applications are also used. Oxidants are usually fed continuously to lessen the impact on other paper-making chemistries.

Even though oxidant demand is important in fresh water treatment it becomes an even bigger factor in process water treatment. For example, if the mill has a fairly closed water system this can greatly increase oxidant

Factors that affect oxidant performance - pH

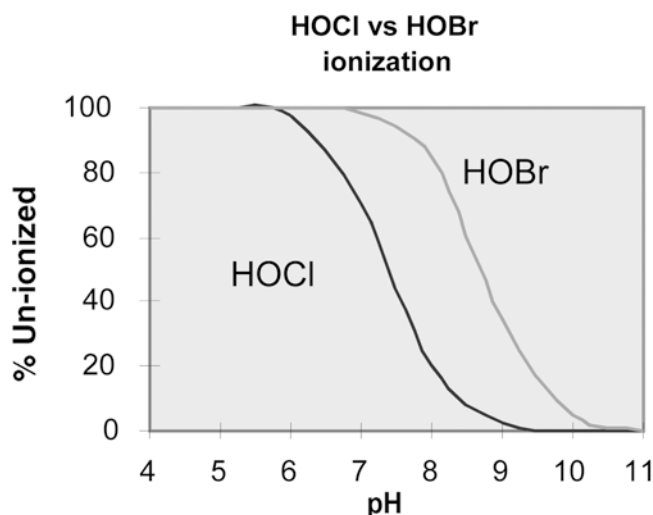


Figure 13 Influence of pH on dissociation of HOCl and HOBr.

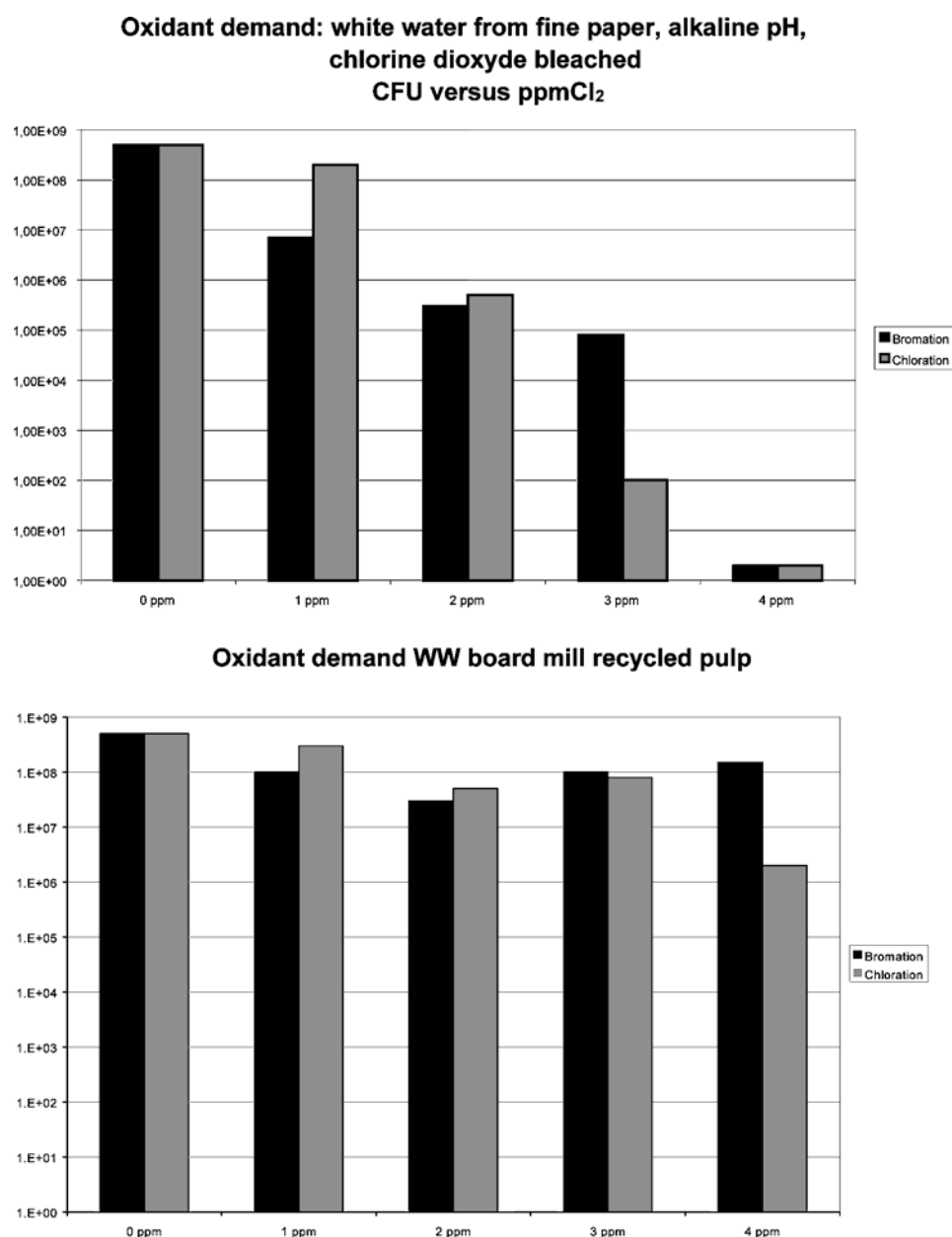


Figure 14 Oxidant demand versus furnish.

demand and consumption. Furthermore, if the mill is using reductive bleaching chemistry this can also greatly affect oxidant consumption.

When using oxidants on the process side other factors come into play. For example, oxidants can react with dyes causing an increase in dye usage. Therefore, oxidants more friendly to dye usage (e.g., PAA) may be preferred over halogenated oxidants. In some cases, oxidant effects may be so detrimental that they may not be allowed (e.g., on coating/release aids and Yankee dryer in tissue manufacturing).

Conclusion. When considering an oxidant program one must look at the whole picture. This includes cost, compatibility with other chemicals, and concerns about corrosion, felt life, or other potential problems. This could justify the use of a more expensive oxidant (e.g., PAA) over cheaper commodity oxidants (e.g., bleach).

5.15.2.2.9 Monitoring oxidant treatment. Halogen containing oxidants are frequently measured using DPD (N, N-diethyl-phenylenediamine) which reacts with oxidants to form a red color. The free Cl₂ test measures HOCl and OCl⁻. The total Cl₂ test measures free Cl and combined chlorine (e.g., chloramines). HOBr can also be measured with the DPD test. If you need to distinguish HOBr from HOCl add glycine (0.2%) to the sample. When HOBr reacts with glycine it can still be measured with the free Cl₂ test but HOCl will not. Stabilised HOBr can be monitored by measuring total Cl₂ or a 4 min free Cl₂.

ClO_2 can also be measured with the DPD test. Multiply the ppm $\text{Cl}_2 \times 1.9$ to get ppm ClO_2 . As with HOBr testing, glycine can be added to eliminate the contribution of HOCl to the reaction. A chlorophenol method is also reported to be specific for ClO_2 measurements.

Dipsticks can be used to measure PAA and peroxide but beware of interferences from other oxidants (e.g., chlorine). The strips will provide a range (e.g., 5–10 ppm). A special strip reader can give more accurate measurements.

On-line testing can be used to measure Cl_2 residuals. Several manufactures now have automated systems utilising the DPD test. These systems can be used as controllers for oxidant dosing. Automatic control of some oxidants can also be accomplished by measuring oxidation-reduction potential (ORP). A number of suppliers sell ORP controllers.

5.15.2.3 *Bio-dispersants on short loop treatment of paper machines*

Paper machine fluids create an ideal environment for the growth of deposit-forming bacteria and fungi. In addition to the highly diverse microflora, the deposits may contain wood fibers, fines, calcium carbonate, pitch, latexes, clay, or other papermaking additives. When deposits break loose and fall into the paper furnish, they result in end product defects such as holes and spots or even paper sheet breaks. Recent interest in less toxic approaches using anionic chemicals like modified lignosulfonates, non ionic surfactants like sulfo succinates or ethylene oxide/propylene oxide derivatives has made use of dispersants an attractive adjunct to deposit control programs in the paper industry. While work has been done to demonstrate the activity of non-ionic dispersants against purely biological films in cooling water systems, the activity of these agents has been poorly understood in the more chemically complex paper processing systems.

Conventional wisdom has held that dispersants act as “biopenetrants” to open the biofilms and allow penetration of the exopolysaccharides by biocides. Claims have been made that the dispersants act to allow penetration of the biocides into the cell or that the dispersants trigger biofilm sloughing. At best the mode of action of these dispersants is poorly understood. Among the difficulties of work with non-toxic agents is the lack of measurement systems. Evaluation of biocides is done by measuring kill or inhibition. With non-toxic materials, alternative methods of assessing chemical efficacy must be developed.

Experimental methods that have been developed to evaluate bacterial biofilms and biofilms containing particulate calcium carbonates. These include environmental scanning electron microscopy (ESEM), Confocal Laser Scanning Microscopy (CLSM), and light microscopy in addition to gross diameter measurements. Chemical agents evaluated include various lignosulfonates and non-ionic dispersants.

In parallel to lab experiences, optical fouling monitors and coupons (removed at 14 days intervals and analysed for colony forming units of aerobic bacteria and sulphate-reducing anaerobic bacteria) have been used to monitor and measure biofilm formation on machines.

In lab studies, lignosulfonates did not appear to inhibit the formation of bacterial biofilms and even lignosulfonate fractions containing sugars appeared to increase biofilm formation.

Non-ionic dispersants inhibited biofilm formation when added to the medium at the time of inoculation. When films did form, they tended to be thinner and less dense than the untreated biofilms. Addition of non-ionic dispersants to existing biofilms did not trigger sloughing or film removal.

Increased amounts of non-ionic dispersants were needed to prevent or inhibit biofilm formation when particulate calcium carbonate was present in laboratory studies but the non-ionic dispersant showed excellent inhibition of deposit formation in a field trial.

Conclusion. Although the idea of removing toxicants from a paper mill deposit control program is appealing, this should not be done without careful consideration of all safety factors. For example the uncontrolled growth of anaerobic bacteria in chests has been implicated in injuries and deaths. Hydrogen, methane and hydrogen sulphide are produced by these organisms.

What about the fact of making aerosols with highly contaminated water near machine showers, high speed machine wet end or around tissue machines?

Spoilage of chests, fiber strength degradation, and starch degradation can occur when the growth of micro-organisms is not controlled. More and more odor problems linked to anaerobic development with sulphides formation and volatile fatty acids are observed (with negative ORP in most of the circuits).

The judicious use of biocides in addition of bio-dispersants to control the adverse effects of microbial activity makes sense for environmental, safety and economic reasons.

5.15.2.4 *Enzymatic slime control* (Steven Tseng, July 1997)

This technology is working with stabilised enzymes based biodispersants specifically formulated for use in paper machine short white water loops.

Several concepts are key to understanding the enzymatic removal of biofilm:

Biofilm [I, 5.1.]. A biofilm is an organic film containing microorganisms embedded in polymeric substances. Biofilms typically consist of water, microorganisms, extracellular polymeric substances (EPS), embedded particles and debris, and dissolved substances. The unchecked development of biofilms on paper machines can result in sheet defects, breaks, malodours, and Microbiologically Influenced Corrosion (M.I.C.).

Traditionally, the development of biofilms is controlled through the application of biocides and deposit control polymers. A number of alternative means of controlling biofilm development have been investigated over the years including the use of enzymes to disrupt the binding matrix of biofilms.

Enzymes. Enzymes are proteins that act to catalyse specific reactions. They help reactions occur, without being consumed by these reactions. Enzymes are named for the reactions that they catalyse or for the substrate that they bind, by the addition of the suffix -ase. For example, cellulase acts to breakdown cellulose.

Typical enzymes investigated for assisting with biofilm removal include: proteases: act to hydrolyse peptide bonds in proteins and peptides, amylases: act to cleave glycosidic linkages in starch or glycogen, levan hydrolyases: act to hydrolyse levan (polysaccharide).

Specifically, the treatment is composed of:

1. At least one acidic protease or alkaline protease.
2. At least one glucoamylase or alpha amylase.
3. At least one surfactant, preferably anionic.

This treatment is preferably composed of all three components in equal proportions. It is recommended that the solution be added to industrial water at a dosage such that each component achieves a concentration of 20 ppm. This dosage level is claimed to result in a significant decrease in biofilm within two to three hours. Higher or lower dosages will affect the time within which effects are noticeable.

Enzymatic slime control. A few paper machines are running using enzymatic blend for biological control. But the potential applications are restricted by the following limits:

- It is absolutely critical that the white water chlorine residual be kept below 0,1 ppm residual halogen. Chlorine will kill the enzymes.
- The proteases are very specific and can only work on specific biofilm. The variations of a paper machine (various pulps, recycled fibers, additives and paper grades changes, fresh water quality) make it difficult to still have the same polysaccharide in the biofilm.

5.15.2.5 Mold proofing treatment of finished paper

(ONDEO NALCO Team Bio Newsletter – Karen R Edwards, contributor: Brian Moran, February 1999).

Application of nonoxydising biocides in papermaking.

What is mold proofing? On occasion, fiber-based products that are resistant to fungal growth are desired. The process that uses chemicals to treat the fibers in order to prevent fungal growth is referred to as mold-proofing. Those paper products include dry-lap, soap wrap, and molded pulp products like flower pots and apple trays. However, wet lap pulp is the most common application. The high moisture pulp is ideal territory for fungal growth. Besides the unsightliness of mold on incoming dry and wet lap pulp, it can cause strength loss, brightness loss, drainage problems and worse of all system inoculation!

Chemical products used for mold-proofing can be applied to the surface of the paper or board at the size press, coater, water box or some other surface treatment. The obvious advantage here is 100% retention of the mold-proofing agent onto the product and better efficiency. The disadvantage is exposure to biocide and in some cases no such feed point exists. Biocides may also be added to the pulp slurry. In this case, the product or blend of products must adhere to the fibers. This is referred to as the product's substantivity.

Products. Water soluble emulsion of 2-(Thiocyanomethylthiobenzothiazole), commonly called "TCMTB" [II, 15.11.] is usually used for mold proofing. Two outstanding features of TCMBT are its substantivity to fiber and fungicidal efficacy, making it a good fit for mold-proofing applications. A 30% emulsion of TCMTB is used in many applications as a fungicide in pulp, paper, and paperboard mills. In addition to being an effective mold-proofing agent, it can be used to control fungi and SRB's in pulp slurries and process water streams. It is effective over a broad pH range and contains no hazardous solvents.

An aqueous solution of carbamate [II, 11] which is effective under both alkaline and acid conditions is effective against algae, bacteria, fungi, and pink slime. However, the carbamate chemistry does not adhere to

fibers. For this reason, it is fed in combination with an aqueous solution of quaternized alkyl dimethyl benzyl ammonium chlorides [II, 18.1.2.]. This chemistry is typically called a “quat” biocide. It is cationic by nature making it very substantive to fiber. It is effective over a broad pH range and although this is primarily a killer, it can function as a preservative as well, depending on dosages and environment. NALCO 7648 is formulated with dispersants and scale control agents to provide effective control of deposit-causing microorganisms. This product is effective against aerobic bacteria, fungi, and spore forming bacteria.

A new fungicide product was developed that shows good promise in mold-proofing applications. The product, is a 4.25% microemulsion of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one [II, 15.5.]. This product is substantive to pulp fibers, allowing for its pulp slurry application for mold proofing. In addition to being an extremely effective fungicide is also effective against bacteria, algae and yeast at low use rates. It is also compatible with oxidising biocides and is odor-free. Recently it replaced a mold-proofing program using a combination of TCMBT and MBT (methylenebis thiocyanate). This solution resulted in lower heavy metals in the effluent and lower overall biocide cost per ton.

Evaluation of mold proofing programs. In order to test the efficacy of mold-proofing programs, samples must be challenged with a fungal spore suspension and incubated under appropriate conditions to determine whether or not the treated material will support fungal growth. Mold-resistance testing can be performed by the supplier Paper Service Lab. This procedure is a modification of ASTM Method G21-70 (Pulp and Paper Chemicals TPC-3.21 Mold Resistance Testing). In summary, four stock cultures of identified fungi are grown in pure culture and the spores are harvested. A spore suspension is prepared, containing a known number of fungal spores. This suspension is used to inoculate the surface of the test material. Control paper is also inoculated to ensure that the spore suspension is healthy. Test material is examined after three days incubation and again at each seven days interval for a minimum of 21 days. Growth observed on the surface of the test material is rated on a scale of 0 to 4, with 4 indicating the heaviest growth.

Similar tests (TAPPI, AFNOR) are also performed to check the mold proofing efficiency of paper treatment for label paper, soap wrapping.

5.15.2.6 *Catalase control in papermaking*

(ONDEO NALCO Knowledge Management System – L. Robertson/L. Hutchinson, August 2002).

Many de-inking facilities bleach fiber using peroxide. Although peroxide is biocidal at very high doses (5000 ppm), many bacteria produce catalase that degrades peroxide into oxygen and water. Is peroxide degraded by this microbial enzyme, the mill may see variation in brightness, as well as increased chelant and sodium silicate use. Typical biocide used for catalase control is glutaraldehyde, alone or in combination with other biocides (quat, THPS).

5.15.3 Monitoring microbial populations and screening efficacy of biocontrol agents

Introduction. To operate a successful microbial control program, it is essential to use standard field methods for evaluating microbial populations, screening and selecting biocides that will be effective in controlling microbial populations in the paper system. This section outlines, in detail, field microbiological methods used to monitor bacterial populations and to measure their response to biological control measures.

The test methods include:

5.15.3.1 *Lab analysis*

5.15.3.1.1 On site and lab spot testing. Identification through on site analysis is a very useful tool because although speck and spot tests are not quantitative and not all components can be identified, it provides answers regarding the composition of deposit material in a short amount of time.

The following guides are helpful in systematically determining the nature and potential cause(s) of the deposit.

5.15.3.1.2. 1% solids content. Measure the weigh of a deposit sample after drying at 105°C in oven and examine the residue to check if it is tacky, or containing ashes, fibers or no residue.

5.15.3.1.3 Speck and spot tests:. Numerous simple tests are available to identify: rosin, lignin, sulphides, wet strength and immediately detect on site the source of the defects in paper:

- Lignin: red coloration with phloroglucinol;
- Pitch: reaction with methylene chloride;

- Aluminium: yellow complex with Morrin dye;
- Carbonate: effervescence with acid;
- Iron: complex formation with ferrocyanide;
- Rosin: raspberry coloration with sugar/H₂SO₄ ;
- Slime: ninhydrin test;
- Starch: blue coloration with iodine;
- Wet strength (UF, MF): red color with FeCl₃/phenylhydrazine test.

5.15.3.1.4 Chemical analysis on acidified sample. If gas evolves during acidification: Moisten lead acetate paper and place over cell. A brown color is a positive test for H₂S (a sign of sulphate-reducing bacteria). Negative test suggests gas is probably CO₂ from CaCO₃. When possible run iron, alumina, barium sulphate tests.

5.15.3.1.5 Ninhydrin spray for bacteria. This test will make a violet coloration with slime but be cautious because it will also react with protein, amines, wet strength agents and be modified with some enzymes.

5.15.3.1.6 Microscopic observations. Very useful on site to identify biological contamination. First examine the sample without dye to see living contaminants (if fresh deposit) and then use dye to improve contrast and better identify slime and bacteria:

- observe at 100× and 400× .
- look for fungal filaments, freshwater organisms, pitch.

100×

Fibers

Pitch bundles

400×

Fillers

Fines

Filamentous Bacteria

Single-cell Bacteria

Fungal filaments

Algae

Protozoa

5.15.3.2 Bioaudit

Key for successful treatment is a good knowledge of the process to be treated and to run an in-deep survey is strongly recommended.

For this survey it is important to:

- Get the process data;
- Get the process flow sheet;
- Check the machine contamination.

5.15.3.2.1 Process data. The influence of several parameters is so important on biocides effectiveness that it is essential to know:

- Physical properties of white water like pH, ORP, temperature;
- Chemical properties: source of the pulp and eventual residual bleaching agent (peroxide, hydrosulfite);
- Paper grade and all additives;
- Fresh water source, consumption, treatment and composition (hardness, manganese. . .);
- Volume of short circuit;
- Coated broke.

5.15.3.2.2 Process flow sheet. This will help to see how the system can recontaminate itself through recirculation of water for showers or dilution. It will help to evaluate storage time and concentration of biocides that can be expected after slug dosage.

In addition some computer assisted calculations will help to simulate the dosages of the product and see if they are compatible with lab study (MIC, killing tests). Other simulation can be carried out using lithium chloride and measuring by atomic absorption its dilution with time in the circuits after a shock dosage.

In any case, it is important to physically examine the circuits to check if they are the same as those shown on drawings. Dead areas like badly agitated tanks, cut piping or piping no longer in use, can severely contaminate the paper.

5.15.3.2.3 Machine contamination. To be sure that the correct problem is addressed, all the deposits on the machine have to be examined through a microscope to identify the presence of fungi, filamentous bacteria, bacteria in zoogaea, nematodes and protozoa. This will help to solve problems linked to fresh water or sprayed water. This is why areas around showers are critical for such examination and success of biocide treatment. Remember that filamentous bacteria don't grow on Petri dishes and are easier to detect using microscopy.

Another potential source of microbial contamination that could lead to poor performance of the machines are the additives. Low bacterial counts are essential in starch, retention polymers, slurries, coating color and size.

In case of bad smell troubles, count of anaerobes and sulphite-reducing bacteria are useful as well as measures in lab of VFA (Volatile Fatty Acid).

5.15.3.3 Biocide screening methods

5.15.3.3.1 Biocide screening method for paper machines.

Why to do toxicity testing? Not all products work equally well in all systems. Some work well as inhibitors, others as killers. Toxicity testing gives a list of products to consider when designing a program.

First rule out biocides that are not compatible with the machine parameters e.g. temperature, pH, redox potential, source of contamination, impact on paper quality (smell, brightness), agreement (food packaging, cigarette paper).

Then run toxicity and killing tests to determine the MIC (Minimum Inhibition Concentration) and identify the most effective biocides.

These evaluations can be done using toxicity tests based on reductase test ("Minitox") or ATP measurement ("Tracide") and plating methods as Petri dishes.

Toxicity measurement: this is a rapid test for biocide product selection, using resazurin.

5.15.3.3.2 Bag kill study: classic biocide screening method for additives

(ONDEO NALCO P&P Document Library – Laura Rice/Linda Robertson/Laurence Hutchinson/S. Ramesh, April 2001).

A bag study is a product-screening test method that is used to select biocides for preserving furnishes, mineral slurries (e.g. clay and calcium carbonate), starch solutions, etc. This procedure is commonly used in materials containing high amounts of solids. Larger volumes of material can be used in this procedure and it also allows monitoring of biocide activity from a few hours to a few days. The bag study draws its name from the fact that bags are commonly used for the testing work as they are cheap, convenient, common, sterile, and disposable. Glass or plastic-ware of appropriate volume can be substituted, but doing so adds complexity to the testing.

5.15.3.4 Catalase test

A Catalase Test can be used to determine if the catalase enzyme is present in a system. This test measures the release of oxygen resulting from the decomposition of hydrogen peroxide. The decomposition of H_2O_2 yields oxygen and water. When catalase-producing bacteria are exposed to hydrogen peroxide, oxygen is released. This test is conducted by placing blotter paper that has been saturated with hydrogen peroxide into a test sample. Oxygen that is released from the decomposition of hydrogen peroxide is trapped under the blotter paper, causing it to rise. The float time of filter pads can be used to measure the amount of catalase in the system.

If the Catalase Test indicates that the enzyme is present at significant or problematic concentrations, plate counts should be performed on stock samples prior to bleaching to determine the level of bacterial contamination. Toxicity studies can then be performed to select a biocide program that will control the bacteria.

Regular monitoring with the Catalase Test and/or plate counts will let you know if your program is effectively controlling catalase-producing bacteria.

Note: The fiber float test is simple and quick to run. Keep in mind that the problem must be massive in order for the biocide application to be economical and to justify the program costs.

5.15.3.5 Microscope examination

(Laura Rice/L. Robertson/Laurence Hutchinson/S. Ramesh/J. Lazonby, April 2001).

Microscopes are very important diagnostic tools. They quickly provide information about the composition of a deposit and what is happening in the papermaking system. They are useful for monitoring the microbiological quality of incoming supply water and determining the contribution of microorganisms to deposits or surface "slime". In addition, many freshwater microorganisms are not able of forming visible growth on media used

for traditional plate count methods (e.g. TGE or Petrifilms) and some may require long incubation periods. Therefore, microscopic examination of supply water and deposits is critical to the success of a biocontrol program. Microscopy allows for rapid diagnosis and photomicrographs can be prepared to document observations. Microscopic examination can also provide information related to the viability of microorganisms. The microscope can also be used to gain valuable information about deposits that are not of microbiological origin.

Different types of microscopes can be used to observe microorganisms. Phase-contrast, brightfield and darkfield microscopy are described below and instruction for sample preparation are outlined. When samples are examined, the total magnification should always be recorded. The total magnification can be calculated by multiplying the magnification/power of the ocular lens by the magnification/power of the objective lens. In addition to magnification, resolution and contrast are also important in microscopy. Resolution is the ability of the microscope to distinguish fine details and structures of a specimen. Under the best conditions, the maximum resolution (resolving power) of light microscopes is near $0.2\text{ }\mu\text{m}$. This means it can distinguish two points as separate objects if they are at least $0.2\text{ }\mu\text{m}$ apart. Also, objects must display some degree of contrast with their surrounding medium in order to be observed. The relatively low contrast of living cells can be increased through the use of dyes or phase-contrast microscopy.

Phase-contrast microscopy. Phase-contrast microscopy permits detailed examination of internal structures in living microorganisms without having to fix or stain the microorganisms. This allows for the examination of living organisms. Phase-contrast microscopy utilises a specialised condenser that contains a phase ring. This results in a ring of light that is focused on the specimen. After passing through the specimen, the light passes through a second phase ring in the objective lens. The path of the light that strikes the specimen is altered, while the unaltered light passes straight through. The light finally rays are brought back together to produce the final image with increased contrast. Because the light must pass through this series of rings, it is essential that the phase rings are routinely focused or aligned to ensure a clear image.

Brightfield microscopy. Unstained cells have little contrast with their surroundings and may be difficult to see. Therefore, samples are typically stained to increase contrast between the specimen and the background. Samples can be treated with dyes that bind selectively to the whole cell or to certain cellular components to increase contrast. However, most staining techniques kill cells and fixation of cells to the slide is usually required. These techniques may also alter the structure of the specimen.

Darkfield microscopy. Darkfield microscopy is typically used to examine organisms that are invisible using brightfield microscopy, do not stain well, or are altered by staining procedures. A special condenser is used which contains an opaque disc that blocks light from directly entering the objective. Only light reflected by the specimen is captured by the objective lens. The result is a light specimen with a black background.

Polarized light microscopy. When polarizing lenses are placed between the light source and the stage and between the stage and the eye piece, the lens can be rotated to extinguish most light. Wood fibers will appear bright on crossed polar lens, as will synthetic fibers. Uncooked starch will show a 'Maltese Cross' formation across the starch granule.

5.15.3.6 Oxido-reduction potential

(ONDEO NALCO Knowledge Management System – Laura Rice/L. Robertson/Laurence Hutchinson/S. Ramesh/J. Lazonby)

You need to know the ORP of your system in order to select the right biocide!

Oxidation and reduction reactions involve the transfer of electrons. Oxidation is the *loss of electrons* and reduction is the gain of electrons. An oxidising environment is created when dissolved O_2 is high, or strong oxidising agents are added, such as sodium hypochlorite (NaOCl) or Cl_2 gas. A reducing environment results from the removal of O_2 and the presence of strong reducing agents, such as bisulphite. Common oxidising and reducing agents are summarised in Table 1. ORP indicates the dominant solution chemistry.

Available electrons are measured using an ORP probe via a platinum electrode, to give relative mV readings of oxidising (electron scarcity, +mV) or reducing (electron abundance; –mV) conditions. We are looking for large changes, e.g., +150 mV vs. –80 mV vs. +80 mV.

A good oxidizing program should have an ORP in the headbox in the range of +200 to +300 mV. ORPs are not absolute numbers.

Using a portable ORP meter. Two choices for ORP probes: small pocket meters and portable meters with separate probes. Each is a platinum electrode with built-in Ag/AgCl reference electrode. The hand-held meters

Table 1 List of common oxidising and reducing agents in environment or industry

Oxidising agents	Reducing agents
<i>Natural in Environment:</i>	
Oxygen (O ₂)	Organics (COD); Microbes (BOD)
Sulphur (S)	Fe ²⁺ , Mn ²⁺
	Sulphides (HS ⁻ , H ₂ S)
	Hydrogen gas (H ₂)
<i>Treatment residues:</i>	
Chlorines (Cl ₂ , HOCl, ClO ⁻ , ClO ₂)	Sodium bisulphite (NaHSO ₃)
Bromines	Sodium sulphite (Na ₂ SO ₃)
Hydrogen peroxide (H ₂ O ₂)	Sodium metabisulphite (Na ₂ S ₂ O ₅)
Ozone (O ₃)	Sulphur dioxide (SO ₂)
Permanganates (MnO ₄ ⁻)	FAS (formamidino sulfinic acid; bleaching)
Chromate salts (corrosion inhibitor)	Ferrous sulphate (FeSO ₄)
Nitrate compounds	Borohydrides
<i>Biocide Products:</i>	
Hypochlorite/Hypobromite	Carbamate
Peracetic acid	Thione

are more versatile and more accurate but pocket meters do the job. External standard solutions should be used to test meter performance over time.

Conclusion. The control of Oxidising Reduction Potential is important in microbiological control:

- To avoid interaction or even deactivation of biocides. Residual bleaching agents can completely deactivate the biocidal treatment;
- To prevent growth of anaerobic bacteria, source of smell problems in the mill or in the paper.

5.15.3.6.1 Chlorine residual measurement. Chlorine will react with many things, including microorganisms. All of these materials are part of the 'oxidant demand' and in order to achieve a free chlorine residual this demand must be satisfied. It is necessary to maintain a free oxidant residual with any oxidant program in order for the program to be effective in controlling microbiological growth. This residual can be measured using a variety of methods. It should be noted that all of these methods were developed using 'clean' water and are much more accurate when performed using freshwater compared to paper mill process water. Regardless of the test method, *residual oxidant tests should be performed immediately following sample collection.* Oxidants will continue to react with materials in the sample, consuming the oxidant and lowering the residual level.

Below are brief definitions of the various halogen species that can be present.

Free available chlorine (FAC): HOCl, OCl⁻.

Combined residual chlorine (CRC): chloramines plus chlorinated nitrogenous organic compounds.

Total residual chlorine (TRC): FAC plus CRC.

Free available bromine (FAB): HOBr, OBr⁻, and bromamines.

Free available oxidant (FAO): FAB plus FAC.

Total residual oxidant (TRO): CRC plus FAO.

When measuring chlorine residuals, both hypochlorous acid (HOCl) and hypochlorite ions (OCl⁻) will be included in the 'free available chlorine' measurement.

The most commonly used field method for determination of chlorine residuals is the DPD test (diethyl-*p*-phenylene diamine) and is available in several different measurement kits. Both color comparators and spectrophotometric methods are available.

The amperometric test method is the most accurate, but it is an expensive laboratory analysis that is not suited to field use.

Table 2

Attributes	Pocket meter	Hand-held meter
Price (in US \$)	Cheap (\$100)	Expensive (\$500 and up)
Utility	ORP only	pH, temperature & ORP
Batteries	Short life; hearing-aid type	Longer life
Ease to Clean	Harder	Easier
Submersible	No	Not without available housing

Table 3 Convenient external standards to verify electrode performance

Solution	Preparation (Freshly made!)	MV reading
Distilled H ₂ O	Room temperature; not stirred	+ 150 to + 200
Hydrogen Peroxide (3% v/v)	1/100 dil. in H ₂ O or buffer	+ 275 to + 430
Carbamate	1/1000 dil. in H ₂ O or buffer	–55 to –80
Commercial Standards	Freshly prepared standard	+ 235 mV

DPD methods are used most often. These methods provide simple, economical means to monitor oxidant programs. Although there are many factors that can reduce accuracy. The greatest interference results from oxidised manganese. Manganese will result in a 'false positive'. High levels of monochloramines and dichloramines will also 'bleed through', resulting in a 'false positive'. This phenomenon usually occurs when greater than 6 ppm of chlorine are recovered in the total chlorine measurement. This frequently occurs in process waters that have a Chemical Oxygen Demand (COD) greater than 400 ppm. COD is a measure of the organic material that is susceptible to strong chemical oxidants.

5.15.3.6.2 Bromine free residual measurements. Chlorine and bromine can be detected and differentiated using the DPD test with the addition of 10% glycine. Because most samples will contain a mixture of both free chlorine and free bromine, both species must be measured. Follow the DPD procedure for chlorine free residuals. Perform the test twice, adding glycine to the second sample. The first measurement will provide a total free residual, a mixture of free chlorine and free bromine. The second measurement will consist of free bromine only. Subtract the second measurement from the first to obtain the amount of free chlorine in the sample.

Both HOCl and HOBr will react with glycine to form chloramine or bromamine respectively. Bromamine is more reactive than chloramine and will react with the DPD reagent as if it were HOBr. Chloramine will not react as free chlorine, allowing for differentiation.

HOBr is typically reported in terms of available chlorine, because it is usually measured using a chlorine procedure. In order to convert the measurement into terms of bromine, the result should be multiplied by a factor of 2.25 and reported as ppm bromine. Most bromine applications use a mixture of chlorine and bromine. Therefore, residual values are typically left in terms of chlorine to avoid confusion. Do not report ppm bromine without running the conversion and making sure the customer understands the difference and wants it reported as bromine.

5.15.3.6.3 Chlorine dioxide residual measurements. Most methods for the measurement of chlorine dioxide are considered unreliable. The DPD method lists a procedure for chlorine dioxide, but the reproducibility is poor and the interfering substances are too great for it to be used accurately for paper mill process water. Therefore, the use of the DPD method is not recommended for use with paper process water.

Another commercially available test, the chlorophenol red (CPR) method, can measure residuals over the concentration range of 0.2 to 2.0 ppm, with a detection limit of 0.1 ppm. This method is used in water and waste treatment plants, but its accuracy in paper systems is unknown. Monochloramine and chlorate ions do not interfere with CPR, but chlorite ion concentrations above 1.5 ppm do interfere. In mills that use chlorine dioxide for freshwater treatment, 1.5 ppm levels of chlorite may not be reached, making it possible to measure free ClO₂ residuals. High levels of chlorite can occur in whitewater if the chlorine dioxide is being applied to the thick stock, eliminating the use of this test for process water applications. Free chlorine will also interfere with the CPR method.

5.15.3.6.4 PAA residual measurements. In accordance with label restrictions, PAA can only be applied up to 0.5–1.5 ppm active ingredient (a.i.) of PAA per ton paper. Most conventional methods for assaying PAA lose their accuracy in this low range. The available dipsticks have a test range from 5–250 ppm a.i. PAA.

As a result, peroxide residuals are used to monitor PAA programs.

5.15.3.6.5 Hydrogen peroxide residual measurements. As discussed under the PAA residual measurement section, hydrogen peroxide residuals can be measured using a dipstick.

5.15.3.7 Hydrogen sulphide measurement

Because of the toxicity of hydrogen sulphide (H₂S), a variety of portable devices have been developed to monitor H₂S levels in air. Many of these devices have alarms that alert workers when H₂S levels are dangerously high. These instruments can be purchased from various suppliers. In addition, detector tubes attached to air pumps can be used to measure H₂S levels in air.

There are also a number of colorimetric tests for measuring H_2S levels in water). Most of these tests are based on a reaction that generates blue methylene, which is subsequently detected by its color. Unfortunately, the color of water samples and the presence of interfering substances can interfere with this test.

A semi-quantitative test for measuring H_2S in water utilises Alka-Seltzer, and should be relatively unaffected by color or chemical interference. Keep in mind that H_2S is easily oxidised. Therefore, unless the sulphide is precipitated, any measurement in water should proceed rapidly after sample collection with minimal aeration.

5.15.3.8 ATP based monitoring tests

Microbial monitoring tests.

Introduction. Papermaking processes are variable and complex and system conditions will impact upon the microbiological control program. Therefore, in addition to screening biocides for a specific process system it is also important to have a mean of monitoring the status of a microbiological control program directly in a system. The ATP meter can be used to collect important information on microbial activity and toxicity in papermaking systems. This information is useful in anticipating and solving problems before they become bigger, more expensive problems. Test results are available in minutes, so the efficacy of the control agent and feed strategy can be examined, changes can be made quickly, and test results can be combined with results of microbial densities in the system determined using dilution plating to provide more complete information.

Microbial activity (ATP) test. Managing microbial activity is an important way to achieve control of a papermaking process. Adenosine Triphosphate (ATP) is the biological energy source used by all organisms. The ATP test is designed to measure the level of microbial activity in a process that might be causing problems and uses biochemical substances derived from fireflies, which react with ATP to produce measurable amounts of light. Microbial activity is measured as ATP and reported in "Relative Light Units (RLU's)". ATP is not a cell count. It is another measurement system that relates to microbial metabolism.

The amount of light produced is proportional to the metabolic activity of the microorganisms present. As the activity of the microorganisms increases in customer's process, higher light levels are measured. Microorganisms have different amounts of ATP. Consequently, there are cases where there will not be correlation between ATP readings and other methods such as dilution plating. For example, bacteria growing under anaerobic conditions generate less ATP compared to bacteria growing under aerobic conditions.

Biocide activity (toxicity) test overview. When microbial activity is not adequately controlled, additional information on the status of microbiological control agents in the system is useful for improving control. Measuring sample toxicity can be used to monitor biocide activity. These measurements are useful for optimising chemical dosing to excessive use rates. In addition, toxicity measurements can be useful for monitoring the toxicity of water discharged to a wastewater treatment plant or natural waterway.

Toxicity is measured using a bioluminescent bacterium. This organism emits light that can be measured by the luminometer. This test organism (toxicity reagent) is grown in large batches and freeze-dried for preservation and storage. Before the test is performed, the organism must be reactivated in buffer to an active, light-emitting state. The initial level of light produced by the reconstituted organism is measured. The bioluminescent test organism is then exposed to a process water sample. If the water sample is toxic to the test organism, the organism is inhibited or killed resulting in a reduction in the light produced. The degree of light reduction provides information on the degree of toxicity or biocide activity in the process sample. If the water has little or no toxic, the second light measurement is almost equal to the initial measurement. This test helps to confirm that an effective chemical has been added at an appropriate dosage. Biocide activity is communicated in "Relative Toxicity Units (RTU's)".

Toxicity tests applications. Both the ATP and toxicity tests can be used in a variety of monitoring and troubleshooting applications. As a part of routine testing, ATP and toxicity testing can be performed on survey samples to monitor the efficacy of a biocide program and will also detect fluctuations in microbial activity, which may be due to changes in stock and additive additions that might have previously gone undetected contributing to potential runnability issues. Monitoring can also detect problems with the actual application of the biocide, such as plugged lines and empty containers, preventing problems before they occur.

When used as part of a cycle study where samples are collected over time from various points within the feed cycle, toxicity tests can monitor the effects of a biocide in your system and the efficiency of the feed strategy. Using this information, the biocide program can be optimised. This information can also ensure that the biocide is circulating throughout the system and is having an effect on the microbial activity present.

ATP and toxicity can also be useful in the biocide screening process. In addition to plating to monitor the effects of a biocide on microbial densities, toxicity and microbial activity can also be measured. Biocide demand

curves generated using ATP can also be valuable in estimating concentrations that might be required for adequate microbiological control in the system.

Biocide Feed-Cycle Studies determine:

1. Adequate biocide is fed into process;
2. Adequate biocide is applied throughout the process segment (during normal conditions);
3. Biocide provides required performance throughout the process segment (during process changes).

5.15.3.9 Optical fouling monitor

(ONDEO NALCO Knowledge Management System – Laura Rice/L. Robertson/Laurence Hutchinson – Contributor: R. Wetegrove).

Introduction. A variety of factors impact the length of time a papermaking process can operate before scheduled or unscheduled maintenance is required. These include the type of product being manufactured, felt life, and control of microbial growth and deposit formation. Papermaking processes are variable and complex. Production rates and product quality can be severely impacted by the presence of surface deposits. Optical fouling monitor (OFM) can be used to monitor surface deposition on-line in process water, providing valuable information on the performance of microbial control programs. The OFM is an on-line device for measuring and recording the degree of soft-deposit accumulation on a transparent test disk that is partially immersed in a flowing process side stream. Successful in-process monitoring of deposit-forming tendencies helps in preventing, anticipating, or solving deposit problems. See Fig. 15–16.

Papermakers operate their process to optimise production, quality, and cost objectives. Therefore, it may be necessary to monitor and control surface deposition to allow for an appropriate period of operation prior to maintenance and cleaning. During this period of operation, deposits accumulate throughout the whitewater loop in the process. A stream of whitewater is continuously pumped through the OFM, so deposition measurements can be made. The unit of measure is the “fouling index”. The fouling index is measured every hour so that trends can be established. These trends can be used for the following objectives:

- to confirm that the microbiological control program is preventing or slowing surface deposition in order to reduce product rejection rates, sheet breaks, and maintenance shutdowns;
- to outline performance improvements that helps the customer to meet new business objectives such as fewer rejects, fewer sheet breaks, or longer production time between scheduled maintenance.

OFM fouling trends need to be established for individual papermaking systems. Although the degree of correlation with rejects and sheet breaks has not been statistically established, experience has shown that it does help guide decision-making and implementation of corrective actions. The OFM can be used to monitor the following program and process variables that affect customer profitability:

- effectiveness of a deposit control polymer;
- biocide efficacy;
- effects of process changes such as pH, temperature, filler loading, furnish components;
- effects of program changes.

Optical fouling monitor operation. The optical fouling monitor measures the amount of fouling and the rate at which fouling occurs. The data is logged hourly and stored in the on-board memory to provide quantitative data reflecting deposition trends of the paper process waters during the testing period. The data can be downloaded using special software.

The monitor measures and records the degree of deposit accumulation on a transparent test disk that is partially immersed in a flowing process side stream, typically whitewater. Hourly, the disk is rotated out of the process water stream into an optical chamber. Once positioned, optical density measurements are made at seven locations on the disk. These positions are continuously in contact with the process stream that flows through the trough. The amount of light that passes through the disk is inversely proportional to the degree of surface deposition. After the measurements are made, the disk is returned to the flowing process stream.

Data interpretation. “Acceptable” and “Unacceptable” fouling indices are specific to each mill situation. When the fouling index has a value of zero, no surface fouling has occurred. A fouling index of 3000 indicates heavy deposition. Fouling index values between 600 to 800 may be acceptable for one location, but unacceptable for another. Data must be collected for an extended period of time to establish normal trends of production conditions that can be used as guidelines for control. The figure below demonstrates the impact of a P.A.A. program on surface deposition. Before the program changes, heavy fouling was observed within 2 to 3 days. Measurements made with the OFM correlated with visual observations of machine surfaces. After the

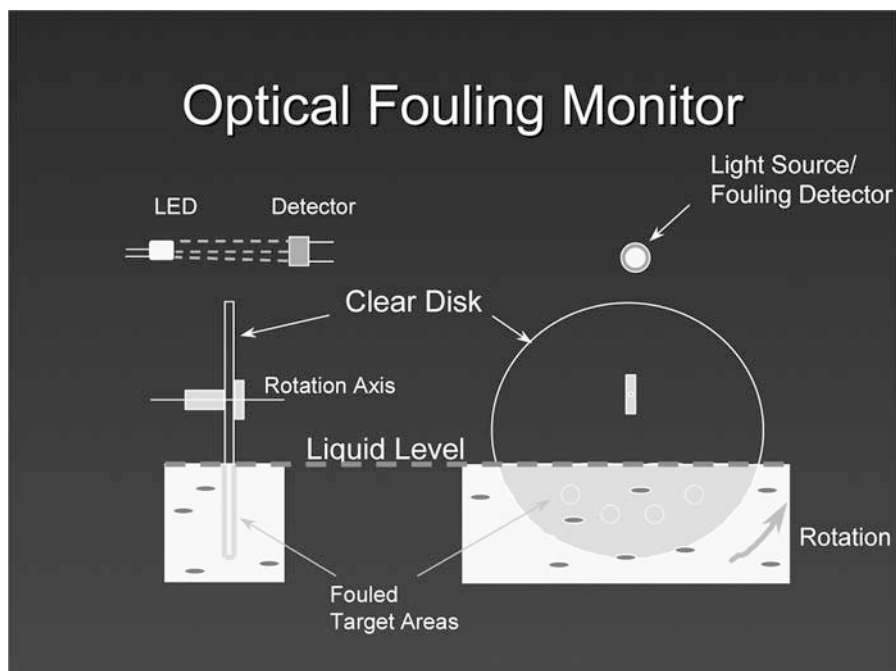


Figure 15 Optical fouling monitor general description.

implementation of the P.A.A. program, fouling did not occur as rapidly and did not reach pre-P.A.A. levels which corresponded to inefficient production and product quality.

Remember OFM data only show the degree of fouling and fouling rates in the test water. If you collect machine performance data such as hole counts, non-mechanic breaks, etc., and relate these data to OFM data, the OFM becomes a more powerful tool for monitoring performance of microbial control programs.

5.15.3.10 Microbiological growth media

(ONDEO NALCO Knowledge Management System – L. Robertson, April 2001).

There are many types of biological growth media commercially available. The Pulp and Paper Research Group has selected media for the cultivation of a variety of microorganisms typically found in Pulp and Paper Systems. Media recommendations for different microorganisms are outlined below.

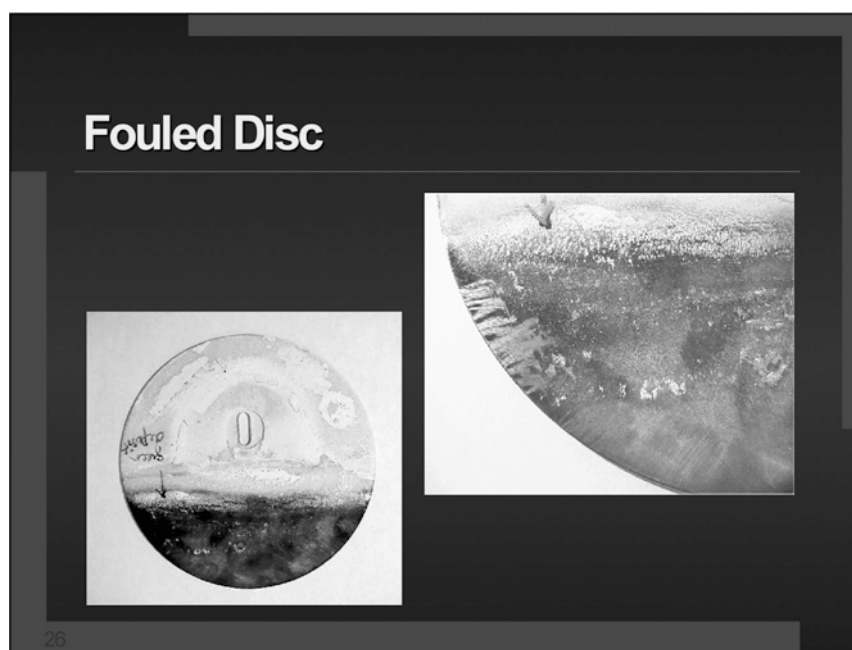


Figure 16 Picture of the disk after a slime deposit.

Aerobic bacteria.

- Tryptone Glucose Extract Agar (TGE) is an agar-based medium commonly used to enumerate aerobic bacteria. The agar must be melted and cooled (48°C/118°F) prior to use.
- Petrifilms are convenient, small media ‘cards’ that are impregnated with a dehydrated growth medium (PCA) and a dye for easy detection of bacterial colonies. Petrifilms should be stored in the refrigerator and are ready-to-use.
- Plate Count Agar (PCA) is an agar-based medium used for the enumeration of aerobic bacteria in finished paper. The agar must be melted and cooled (48°C/118°F) prior to use.

Anaerobic bacteria.

- Thioglycollate is an agar-based medium commonly used for the enumeration of anaerobic bacteria. It contains an indicator dye (methylene blue) that is green when exposed to oxygen and yellow/tan under anaerobic conditions. This agar must be melted and cooled (48°C/118°F) prior to use. The use of an anaerobic incubation chamber (e.g. AnaeroPack) is also required for the enumeration of anaerobic bacteria.
- SRB Tubes contain a medium developed at Nalco for the selection of anaerobic bacteria called Sulphate-Reducing Bacteria (SRB). This medium was designed to provide an anaerobic environment, eliminating the need for specialised incubation equipment. The tubes must be melted and then cooled (48°C/118°F) prior to use.

Fungi.

- RK Agar was developed for the growth and enumeration of yeast and mold. This medium is specifically designed to inhibit bacterial growth and restrict fungal colony size for ease in counting. However, bacterial growth may occur and “mucoid-type” colonies should be examined microscopically to determine if these colonies are comprised of bacteria or yeast. RK Agar must be stored and incubated in the dark. The dye used to inhibit bacterial growth is light sensitive and can become toxic even to fungi if it is left in the light. Plates should be incubated for 5 days prior to counting.
- Yeast & Mold Petrifilms are also available from 3M. However; Nalco’s Pulp and Paper Research group has determined that results are sometimes inconsistent when used for pulp and paper process samples. Please use with caution. Many bacteria are able to grow on the yeast and mold Petrifilms.
- Potato Dextrose Agar (PDA) is an agar-based medium commonly used for the growth and enumeration of fungi. The agar must be melted and cooled (48°C/118°F) prior to using. The addition of tartaric acid is necessary to minimise bacterial break through. Plates should be incubated for 5 days prior to counting.

5.15.3.11 Volatile fatty acid (VFA) measurement

(ONDEO NALCO Knowledge Management System – Laura Rice/L. Robertson/L. Hutchinson – Contributor: B. Urtz, May 2001).

The most accurate way to monitor Volatile Fatty Acids (VFA) that can cause odor/odour problems in the finished sheet is through the use of gas chromatography (GC). GC can measure individual VFA in water or paper samples. The analysis includes measurements of acetic, propionic, and butyric acids. These three acids typically account for ≥98% of VFA in process water samples containing high VFA levels. If there is a need to measure other VFA (e.g. pentanoic acid) this can be done for an additional charge. Before a sample is sent for analysis, it is recommended that the sample be preserved by acidification to pH 2–3 to prevent further VFA production in transit.

For on-site analysis, field test devices are available that can be used to monitor total VFA levels and/or VFA trends. The test is relatively cheap and easy to use. However, in addition to the reagent set, other supplies are required including a boiling water bath and a spectrophotometer. Before the on-site test is relied upon to any great extent, a comparison should be made between the test and GC. In some mills the test will overestimate VFA levels due to a lack of specificity with the test. However, even in these mills the test may be useful in monitoring VFA trends. Keep in mind that the volatile acids test will react with organic acids besides Volatile Fatty Acids (VFA) and therefore, overestimate VFA levels in some situations. The real strength of the test is in monitoring VFA trends.

5.15.4 Equipment

Safe handling of biocides. All chemicals should be handled cautiously (it is prudent to minimise all chemical exposures), but biocides require special caution because they are designed to be toxic to living organisms.

Biocides in the United States have gone through a long registration process with the Environmental Protection Agency. As part of biocide registration, a very specific label is developed. Included on this label are statements regarding:

- The hazard to humans,
- Environmental hazards,
- Storage and disposal,
- Practical treatment,
- Directions for use.

All biocide users should be well informed regarding safe handling, storage and feeding of these products. Make sure that proper safety equipment is used when feeding, and that eyewash stations and showers are available. Make sure bulk tanks are properly labelled. A Material Safety Data Sheet (MSDS) should be kept with the product at all times. The MSDS explains how to handle the product and what to do in case of an accident. The Nalco Alert telephone number should also be posted with the MSDS. Additionally, registration labels should be kept on storage containers at all times.

Never eat or smoke when working with biocides.

Never wipe your eyes with a clothes, gloves or hands that have contacted a biocide. Always read the MSDS before working with a new product.

Protect your eyes with a face shield or goggles: Eyes are particularly sensitive to chemical products.

Wash your hands after working with biocides.

Following are some ideas on the safe use of biocides.:

- Locate biocide containers and pumps in areas where there will be little chance of human contact.
- Consider the temperature of the storage area, proximity to other products, capability for effective spill containment, ventilation and location of emergency exits when selecting the storage site.
- Carefully label all containers and tubing that carry biocide.
- Distribute an MSDS to key personnel and post one beside the product. Post the supplier alert telephone number and develop an emergency action plan.
- Locate showers and eyewashes near the biocide containers and pumps, and have face shields, gloves, aprons, boots, biocide neutralisation chemicals, etc., nearby.
- Use quality pumps, timers and tubing. Do not add biocide by hand with containers.
- Read the equipment operating manual before beginning to feed the biocide.
- Interlock the electrical power going to the pump with the machine system to prevent pumping biocide when the machine is down.
- Inspect pumps and transfer lines often to ensure they are maintained, and keep the area clean. See Fig. 17–18.

Feeder BCDMH

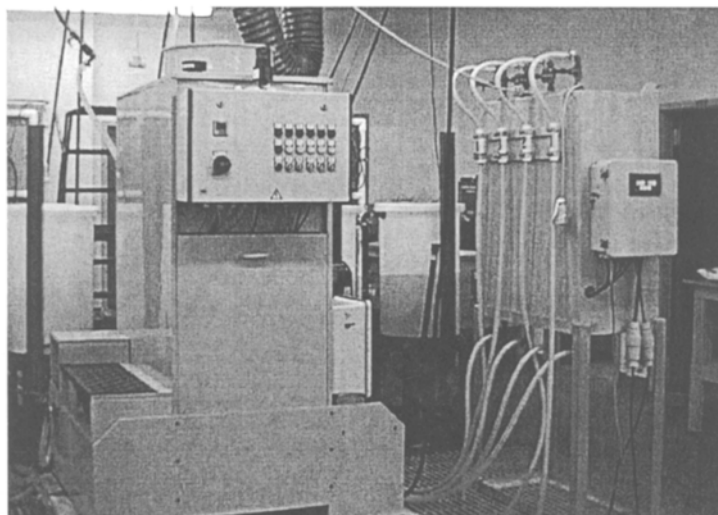


Figure 17 Feeder for BCDMH powder.

Safe feeding equipment. Do not use feed methods such as drum tipping, bucketing and feeding with metering pumps because they invite safety problems. Drum tipping and bucketing are the most dangerous methods. The operator is clearly susceptible to eye or skin contact as well as to breathing hazardous vapours.

Metering pumps are safer, but may pose hazards. Since the pump discharge is under pressure, pumps can potentially leak or spray chemicals. When pumps break down, someone has to fix them. There is no easy way to rinse out a broken pump until it is apart. The repair technician will be exposed to the biocide.

Eductor feed systems are recommended for feeding liquid biocides. The eductor feed systems are designed for simple, safe operations without manual handling or operator exposure to the product. Eductors use the “Venturi” principle. They contain no moving pump parts that would need repair or maintenance, and the lines carrying concentrated products are under negative pressure, preventing leaks or chemical spray. The pressurised portions of the feed system (drive water) carry just make up water with low concentrations of chemical that would normally be regarded as safe feeding system.

The system includes a positive displacement (PD) metering pump, dilution water piping and a control panel for system logic. The control panel has a 24-hour timer to set cycles. Upon completion of the feed cycle, the system flushes the feed line with at least three pipeline volumes of water. Dilution and flush also run off the timer. This automatic flushing system reduces the risk of exposure to potentially harmful concentrations of chemical, making it much safer than a simple metering pump.

5.15.5 Safety

(ONDEO NALCO Knowledge Management System – Environmental Health & Safety G. Horacek, Geak Lian Chia, March 2001).

5.15.5.1 Safe handling of biocides

All chemicals should be handled cautiously. It is sensible to minimise chemical exposure. Biocides often require special caution because they are designed to be toxic to living organisms. Even so, biocides are safe to handle and use, provided proper safety precautions are practised and the products are used in accordance with their product data sheets and labels. See Fig. 19.

Biocides in most countries go through an extensive registration process with agencies such as the US Environmental Protection Agency and FDA. As part of biocide registration, a very specific label is developed for each chemical formulation. Included on this label are statements regarding:

- Hazard to humans and animals,
- Environmental hazards,

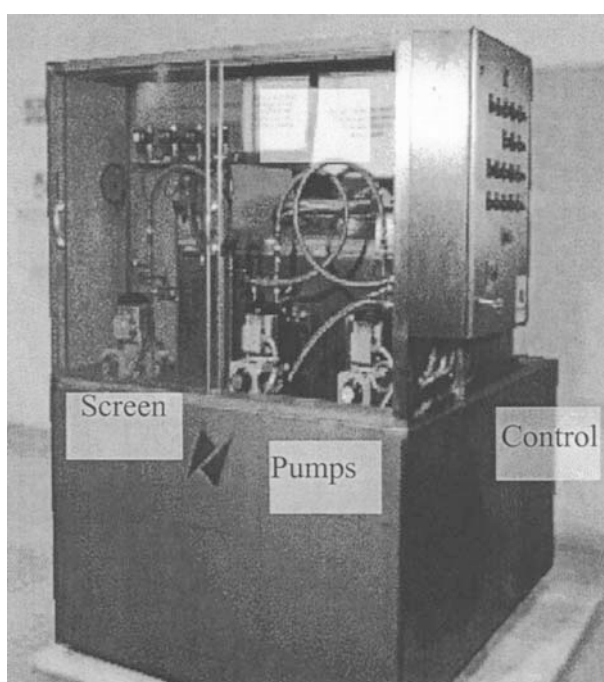


Figure 18 Automatic dosing station for peracetic acid.

exemple: toxicity test of the effluents going to the biological waste water station				
Sample	Initial value	Final value	Relative Toxicity units	Comments
Contrôle	18 000 690	17 827 812		
additive 1 (10 ppm)	18 009 066	15 390 713	0	no toxicity
additive 2 (10 ppm)	18 723 724	15 444 940	0	no toxicity
OBA (10 ppm)	18 640 398	14 757 176	0	no toxicity
water anaerobic station 1	18 457 656	90	203 114	Very high toxicity, should kill any bacteria
additive 3(undiluted)	17 821 034	17 549 824	0	no toxicity
water anaerobic station 2	18 102 092	2 025 490	8	no toxicity

Figure 19 Effluent toxicity measurement.

- Storage and disposal,
- Practical treatment,
- Directions for use,
- EPA registration number.

The directions for use are particularly meaningful because they identify the legally approved applications and the maximum allowable dosages for the product in each application. For example it is a violation of US Federal law to use biocides in a manner inconsistent with labelling. This includes exceeding label limits and applying any chemical to non-label listed applications.

After the label, the next most important documentation required for chemicals is the Material Safety Data Sheet (MSDS). The MSDS should be kept with the product at all times. The MSDS explains how to safely handle the product and what to do in case of a fire or accidental contact/spillage. The MSDS gives environmental data and the status of the product under various regulatory classes.

5.15.5.2 Developing a safe microbiological control program

The ultimate responsibility for safety of chemical applications resides with the end user. However, as employees of Nalco, it is our responsibility to educate our customers in the safe handling, storage and feeding of our products. Part of our responsibility includes:

- Making certain proper safety equipment is used when feeding the material;
- Making certain that eye-wash stations and showers are available;
- Making certain bulk tanks containing the chemicals are properly labelled;
- Posting the Nalco Alert telephone number and the MSDS (above).

Practical program hints include:

- Locate biocide containers and pumps in areas where there will be little chance of human contact;
- Consider the temperature of the storage area, proximity to other products, capability for effective spill containment, ventilation and location of emergency exits when selecting the storage site;
- Carefully label all containers and tubing that carry biocide;
- Distribute an MSDS to key personnel and post one beside the product;
- Post the Nalco Alert telephone number and develop an emergency action plan;
- Provide training to all mill personnel who are impacted by the biocide application;
- Locate showers and eyewashes near the biocide containers and pumps, and have face shields, gloves, aprons, boots, biocide neutralisation chemicals, etc., nearby;
- Use quality pumps, timers and tubing. Do not add biocide by hand with containers;
- Read the equipment-operating manual before beginning to feed the biocide;
- Interlock the electrical power going to the pump with the machine system to prevent pumping biocide when the machine is down;
- Inspect pumps and transfer lines often to ensure they are maintained, and keep the area clean.

5.15.5.3 *Disposal issues*

5.15.5.3.1 *Biocides.* Provisions for biocide disposal are part of a safe biocide application plan. Biocide wastes are toxic and their disposal is regulated. Improper disposal of excess biocide is a violation of federal law. Nalco's Porta-Feed program eliminates most customer concerns about disposal of biocide containers, because empty Porta-Feeds are returned to Nalco for handling. Trial quantities should be used entirely to avoid problems with disposal. When nothing is left, no disposal is necessary.

Small test quantities (e.g., two-ounce samples) of Nalco biocides also require proper disposal. Ideally the samples can be disposed of through addition to mill process waters where the biocides are normally fed. If the mill wastewater treatment system is large (e.g., million gallon/day volumes) it is probably safe to rinse the remains of a laboratory sample into the waste treatment system. Triple-rinse the bottles after emptying. Biocides should not be discharged to the sanitary sewer. Rinsing them into a home septic system could destroy the bacteria that treat your household sewage. The ideal disposal method is to use up the entire sample.

5.15.5.3.2 *Petrifilms and agar plates.* Petrifilms and agar plates can be sterilised through a variety of means. These include autoclaving, incineration in a hog fuel burner or other mill incinerator, or soaking the materials in a strong bleach solution for an hour. The materials can then be discarded.

5.15.5.4 *Program application approvals/registration [I, 4.]*

In the United States, the EPA and FDA have oversight for chemicals used as biocides. Every product sold must have EPA approval; some applications require FDA approval as well (see below). Canada has a separate, but similar program. In Europe, the EC has authorisation/regulation procedures in place. In many cases, BfR (Federal Institute for Health Protection of Consumers and Veterinary Medicine, - the German FDA) approval is required for a chemical to be used. Different oversight procedures are used in the rest of the world. Specific guidance on these regional and/or country specific regulations should be sought from specialists and are beyond the scope of this manual.

5.15.5.4.1 *U.S. environmental protection agency (EPA).* Chemicals or biological substances that are intended to control weeds, insects, fungi, bacteria or other pests are considered to be pesticides. Pesticides are highly regulated since they are capable of affecting organisms other than those for which they were designed to control. They also may have severe adverse effects on man and the environment. Pesticides are regulated by the EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). FIFRA requires pesticides to be registered by the EPA and authorises the agency to prescribe conditions for their use.

FIFRA regulates the manufacture, sale, delivery and use of pesticides and pesticide products. It is basically a labelling law. Successful registration of a product, results in an "accepted" label which is the "license to sell." Registration is product specific, not chemistry specific.

There are two kinds of registrations issued by the E.P.A.; Primary and Distributor. The Primary Registration is acquired by submitting all of the required data and information. This involves forms, product labelling, technical and scientific data, including efficacy and toxicology information. The Distributor Registration is issued to a company who wishes to contract with a primary registrant and agrees to sell their registered product under their own brand name. This procedure limits one's ability to alter or repackage the product, although it is a rapid method of bringing a product to market.

The "Directions for Use" on the product label defines the limitations of using the product with regard to application site and dosage. Even if the product meets FDA requirements for a specific application, but the product label does not list the application, the product cannot be used. There is also the possibility that a competitor may have the same chemical as a Nalco product, but the product labels are different. This is because the EPA approved the product labels which are product specific, but the FDA approved the "chemical" for the application.

5.15.5.4.2 *U.S. food and drug administration (FDA).* Products that are marketed to customers who have a responsibility to comply with FDA regulations must comply with the regulations promulgated under the Food, Drug, and Cosmetic Act (FDCA). This comprehensive Act covers chemicals that are used either directly in or on food such as in sugar beet processing or in the preparation of items that may contact food such as paper or paperboard.

Unlike the biocide regulations under the EPA, the FDA does not rely on product names or labels. Instead, FDA regulations are based upon chemistry only. The Act requires potential sellers of a food additive substance to establish the safety of the additive under prescribed conditions of use. The burden of establishing safety lies with the petitioner and not the US. government. If Nalco was to go through this procedure to get, for example, 176.170 approval for (MBT), this approval would apply to Nalco's 10% formulation (7619), as well as to

our competitors various formulations (not limited to 10%). If a chemistry is approved, it is approved for any concentration or formulation.

The number one question asked about FDA approval is, where exactly can a product be applied and still meet the regulations. The answer to this question is not always clearly defined. For example, Nalco 7634 is approved for Pigments & Filler Slurries up to 300 ppm (active). Does this mean the product can be fed into a coating mix tank which uses pigments, or does the product have to be fed into the pigment make down tank? The answer is as follows. Even though the pigment slurry is going into the coating make down tank, Nalco 7634 cannot be fed directly into the coating make down tank.

To make these types of decisions as simple as possible, the FDA Compliance Sheet can be used to help identify approved feed locations. This approval sheet does not cover every possible application situations, but it can be used with confidence for the more common product uses. In the event the mill would like an official confirmation that a particular feed location is, or is not FDA approved, a letter can be prepared by Nalco's EH&S Department.

5.15.5.4.3 Pacific. The countries of the Pacific tend to use US FDA and sometimes EPA information. Some countries have specific rules that are followed. For example, Korea has the NIER (National Institute of Environmental Research), Australia the NICNASS, however, Indonesia is not regulated and Taiwan has no specific regulations for biocides.

5.15.5.4.4 Europe. Depending upon the location of the country, paper chemical applications may be regulated by multiple agencies. Norway, as well as other Nordic countries, is very stringent about environmental regulations and safety, while France is more relaxed on paper chemical applications. Regulations tend to go hand in hand with public awareness of biocide applications.

Europe has now introduced the **Biocidal Products Directive (BPD)**, which will become the definitive guideline for approved biocidal substances. Extensive technical and eco-tox support is required to ensure that a supplier's product is put onto an approved list. The onus is on the manufacturer of the biocide to provide this data. BPD became effective in 2000, will be fully enforced by 2005. Any product not fully supported must be withdrawn from sale by that date.

Specific European countries. Despite European-wide guidelines, some countries still exercise unilateral control on biocide 'acceptability'.

Sweden – product control board. Supplier must provide full eco-tox package before being granted approval to sell a biocide, or have a letter of access from the raw material manufacturer to their eco-to data, to sell the product in the supplier's name.

Finland – chemicals inspectorate. Approach is similar to Sweden, only registered chemicals are allowed to be sold.

Netherlands – CTB. Similar to Sweden.

Germany. Germany has the government-run **BGvV** (now named BfR) system, where a committee meets twice a year to evaluate products not already on their recommended (approved) list, based on eco-tox data or submissions from approved institutes such as Isega. Specific to food contact paper and packaging but used by industry for many years as a benchmark, until the BPD came along. BGvV § XXXVI specifies which molecules are permitted for use, as well as maximum concentrations allowed in the finished paper, based on a formula of how much may migrate to the food. Different sub-regulations govern packaging for fatty or hot foods.

European market issues. Some industry initiatives, such as Nordic Swan. This is a set of parameters that covers water use, energy use, environmental impact of the paper-making operation, and also covers additives and chemicals. Being able to put a 'Nordic Swan' label on your paper allows it to be sold to a number of otherwise closed markets. Use of specific biocides, having good environmental profiles, is also defined in this guideline.

Safety. Most countries have government guidelines on safe handling and use of chemicals, such as UK Health & Safety Executive COSHH (control of substances hazardous to health), which is more focused on exposure risk. This can cause problems, such as when HSE issued a chemical alert about glutaraldehyde, and its potential for causing respiratory sensitisation. Immediately, several customers asked to change from glutaraldehyde to a different chemistry.

5.16 Microbicides for the protection of textiles

A.W. WYPKEMA

5.16.1 Definition and differentiation of terms

Microbicides are used to effectively preserve textile materials in several ways:

- to keep the textile materials in a hygienic conditions:
 - to significantly limit the incidence of bacteria;
 - to reduce the formation of odour as a result of the microbial degradation of perspiration;
 - to indirectly limit the number of glycyphagus, whose excrements cause allergic reactions to people with sensitive bronchi;
 - to avoid transferring and spreading pathogens;
- to avoid mould formation on the textile materials;
- to avoid decomposition of the textile materials.

The “Lexicon für Textilveredlung” (Rouette, 1995) distinguishes the following types of anti-microbial treatments:

- germicidal treatment. This term is used in combination with anti-rot treatments;
- hygienic treatment. This term is used for the elimination of pathogenic micro-organisms;
- anti-bacterial treatment. This term includes bactericide- and bacteriostatic treatments:
 - bactericide treatment. This treatment aims to kill all bacteria;
 - bacteriostatic treatment. This treatment limits the growth of the bacteria;
- anti-mycotic treatment. This term includes fungicidal- and fungistatic treatments:
 - fungicidal treatment. This treatment aims to kill all fungi;
 - fungistatic treatment. This treatment limits the growth of the fungi;
- algicidal treatment. This term is used for the prevention of algal growth;
- deodorizing treatment. This treatment aims to prevent the formation of nasty smell;
- anti-rot treatment. This treatment aims to protect materials from bacteria and fungi under unfavourable storage conditions, like high relative humidity and temperature.

Recently the “Hohenstein Institute” (Mucha et al, 2002) has drafted the following general definition for antimicrobial activity:

Definition: Antimicrobial activity is a collective term for all active principles which inhibit the growth of bacteria, which prevent the formation of microbial colonies and which may destroy microorganisms.

5.16.2 Legislation

The number of textile materials which have been antimicrobially modified, has increased considerably over recent years. In the past predominantly technical textiles had antimicrobial finishes, in particular to protect against fungi. Nowadays textiles worn close to the body are increasingly being finished and modified in this way. This development is accompanied by:

- an increase in awareness of hygiene issues and demand for hygienic products on the one hand;
- increasing criticism of the use of chemicals on humans on the other hand.

In both the USA and Europe, the decision was taken on a political level to make use of antimicrobial agents subject to statutory requirements. The European Parliament and Council directive 98/8/EC concerning the marketing of biocidal products came into force on 16 February 1998 (EC, 1998): ‘the Biocidal Products Directive’. This directive encompasses general descriptions of the products, physio-chemical, toxicological and ecotoxicological tests as well as additional biological and biochemical checks. The transition period allowed for national implementation of the provisions expired in February 2000.

The question of whether or not textiles with anti-microbial finishes or modifications are subject to the Biocidal Products Directive is crucial. A legal running must be made to decide on whether the qualifying criteria as to whether or not textiles fall under the Biocidal Products Directive, will be based on the benefits or intended purpose of the product.

Biocides used for textiles could be categorised in main group 2: Preservatives, under product type 9, which concerns fibre, leather, rubber and polymerised materials preservatives (EC, 1998). The bill that Germany set up to implement the directive, adopts the exact wording of the definition of the EU directive for biocidal products, but also specifies the area of application, identifying 4 main groups with 23 types of product, including 2 which could affect the textile industry: "Human hygiene biocidal products" and "Protective coating agents".

In Europe, the antimicrobially modified textile materials are anyhow subject to the European Council directive 76/769/EEC on the approximations of the laws, regulations, and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (tin, PCP and cadmium) (EC, 1976). Commission directive 1999/51/EC adapted to technical progress for the fifth time Annex I to this directive (EC, 1999). The directive prohibits the use of more than 0,1% by mass pentachlorophenol (II, 7.5.4.)* and its salts and esters (II, 9.9.), in substances or preparations placed on the market. By way of derogation until 31 December 2008 France, Ireland, Portugal, Spain and the United Kingdom may chose not to apply this provision to substances and preparations intended for use in industrial installations not permitting the emission and/or discharge of pentachlorophenol (PCP) in quantities greater than those described by existing legislation in the impregnation of fibres and heavy-duty textiles not intended in any case for clothing or for decorative furnishings.

5.16.3 Voluntary safety – and enviornmental labels

The award of the European Community eco-label for textile products is subject to Commission Decision 1999/178/EC of 17 February 1999 (178/EC, 1999). The criteria relevant for antimicrobially modified textile materials are:

- Tetrachlorophenol and pentachlorophenol (II, 7.5.) incl. their salts and esters shall not be used;
- The amount of free and partly hydrolysable formaldehyde (II, 2.1., 3.) in the final textile material shall not exceed 30 ppm for products intended for infants of less than 2 years of age, 75 ppm for products that come into direct contact with the skin, and 300 ppm for all other products.

The latest draft of the revision to the above described Decision (Textile Ecolabel Working Group, 2001) extends the criteria for biocidal or biostatic products:

- Chlorophenols (their salts and esters), PCB and organotin compounds (II, 19.) shall not be used during transportation or storage of products and semi-manufactured products;
- Biocidal or biostatic products shall not be applied to products so as to be active during the use phase.

The award of the European Öko-Tex Standard 100 label is subject to the General and special conditions for the authorization to use the Öko-Tex Standard 100 mark (Ökotex, 2002). The conditions relevant to anti-microbial textiles are:

- Pentachlorophenol (PCP), 2,3,5,6-tetrachlorophenol (TeCP) and orthophenylphenol (OPP) (II, 7.4.1.) may not be present in the textile material in concentrations higher than 0,05-1,0 ppm (depending on the substance and the application of the textile material);
- Tributyltin (TBT) may not be present in the textile material in concentrations higher than 0,5-1,0 ppm (depending on the application of the textile material). Dibutyltin (DBT) may not be present in textile materials for baby's in concentrations higher than 1,0 ppm;
- The amount of free and partly hydrolysable formaldehyde in the final textile material shall not exceed 20 ppm to 300 ppm (depending on the application of the textile material);
- Anti-microbially modified textile materials can only be rewarded the Öko-Tex Standard 100 mark if the anti-microbial agent is considered safe "for humans and environment" by the Öko-Tex organisation. The product Sanitized T96-20 from Sanitized Marketing AG, Burgdorf, Switzerland was considered safe by the Öko-Tex organisation in 2002.

5.16.4 Test methods

A distinction is made between two different types of test systems:

- The 'Agar diffusion test' which gives a semi-quantitative result;
- The suspension test or 'Challenge test' which gives a quantitative result.

*see Part Two – Microbicide Data

Table 1 gives an overview of different test methods used to test textiles, fibres, yarns and polymers for anti-microbial effectiveness.

When testing for the specific anti-microbial activity of an active substance, reference samples and control materials which have not been finished are necessary. They must be of the identical structure and chemical composition as the test material, but without the anti-microbial finish. When testing for the general anti-microbial activity at which all textile related activity affects the microorganisms, the reference samples mentioned above are not required; instead, suitable positive growth controls which prove the biological operability of the test or test organisms are required.

The first method which quantifies both the overall activity and the specific anti-microbial activity is the Japanese standard JIS L 1902. Unfortunately in literature a misunderstanding is common where the terms 'overall activity' and 'specific anti-microbial activity' are used incorrectly. However, despite of the confusion between the terms, the Japanese standard enables two different statements to be made concerning the anti-microbial effect of modified textiles. The comparative simple experimental implementation of the JIS L 1902 and an alteration to the evaluation criteria relating to the specific anti-microbial effectiveness and overall effectiveness as described above, together with the presentation of the results in log10 increments, form the basis of a modified Hohenstein (Germany) test method.

The international standardisation organisation (ISO/TC 38/WG 23) is currently developing a complete system of "Test methods for anti-bacterial finished textile products" which will not only cover the wide variety of different materials and modifications available, but also deal with their effects on the skin.

5.16.5 Preservation of textile materials by proper maintenance

By far the most textile materials have no anti-bacterial activity of them selves and are not anti-microbial modified during their production. Still these textile materials should be kept in a hygienic condition and mould formation on – and decomposition of these textile materials should be avoided. In many cases this can be done successfully by proper maintenance.

Proper maintenance means regular cleaning and storing under conditions which are unfavorable to micro-organisms. During cleaning two effects can be reached:

- removal of soil (which can be a feedstock to micro-organisms) and micro-organisms;
- active killing of micro-organisms, e.g. by high temperatures or by the addition of microbicides during the cleaning cycle. Most surface active agents (in cleaning detergents) already work as microbicides mostly by disrupting the cell membrane. Optionally other microbicides can be added during the cleaning cycle in order to secure a hygienic cleaning result. In industrial laundries it is common practice to add chlorine or hydrogen peroxide as a microbicide (II, 21.) during the cleaning cycle. For special purposes (like hospital operating room textile materials) a sterilisation treatment with steam at about 130°C is added.

Storing conditions that are unfavorable to micro-organisms are mainly low air humidity and/or low temperature. Also sealing to avoid micro-organic contamination is a well known method. The actual maintenance scheme necessary for a certain product depends on the product properties, the conditions under which it is used and the degree of hygiene one aims to reach.

Although a proper maintenance scheme is the preferable solution for most textile materials, for many textile materials a proper maintenance scheme cannot be identified or is unpractical. This is the case for products like uniforms, tents and technical textiles such as geotextiles (like sheeting for drainage and soakage pits, sheeting for embedding pipes and standing piers), roof coverings, facade linings, wall paper, bed mattresses, etc..

In these cases there are two options:

- choose materials that are less susceptible to micro-organic growth. Fibers from natural sources are in general more susceptible to microbial attack than fibers from synthetic sources. Fibers from polyvinylchloride have inherent anti-microbial activity.
- modify the surface structure of the textile material in such a way that microbial colonisation of the textile is prevented because the cells are prevented from adhering to the fibre surface. Fibers from polyvinylfluoride or fluorocarbon finished fibres prevent adhering by their low surface tension. Adhesion can also be prevented by the surface structure of the fibre (e.g. the lotus effect or micro-domain structured surfaces. Also the development has been described of anti-adhesive, "intelligent" polymers which prevent the formation of a biofilm on implants and thus offer a preventative measure for infections associated with implants, the so-called plastic infections.
- modify materials with anti-microbial agents.

Table 1 Methods used to test textiles, fibres, yarns and polymers for antimicrobial effectiveness.

Designation	Title	Origin	Principle
SN 195920: 1992	Textile fabrics: Determination of the antibacterial activity: Agar diffusion plate test.	Switzerland	Agar diffusion test
SN 195921: 1992	Textile fabrics: Determination of the antimycotic activity: Agar diffusion plate test.	Switzerland	Agar diffusion test
AATCC 147: 1993	Antibacterial assessment of textile materials: Parallel streak method.	USA	Agar diffusion test
AATCC 90: 1982	Antibacterial activity of fabrics, detection of: Agar plate method.	USA	Agar diffusion test
AATCC 174: 1993	Antimicrobial activity assessment of carpets.	USA	Agar diffusion test
JIS L 1902: 1998	Testing method for antibacterial textiles.	Japan	Agar diffusion test
AATCC 100: 1993	Antibacterial finishes on textile materials: Assessment of.	USA	Challenge test
SN 195924: 1983	Textile fabrics: Determination of the anti-bacterial activity: Germ count method.	Switzerland	Challenge test
XP G 39-010: 2000	Properties of textiles – Textiles and polymeric surfaces having antibacterial properties – Characterisation and measurement of antibacterial activity.	France	Challenge test
AATCC 30: 1993	Antifungal activity, Assessment of textile materials: Mildew and rot resistance of textile materials.	USA	Agar diffusion test, Soil burial test and Humidity chamber test
JIS Z 2911: 1992	Methods of test for fungus resistance.	Japan	Fouling tests
EN ISO 846: 1997	Plastics – Evaluation of the action of microorganisms	International	Fouling tests, Soil burial test
EN-ISO 11721-1:2001	Textiles – Determination of resistance of cellulose-containing against micro-organisms; Soil burial test – Part 1: Determination of a rot resistance treatment.	International	Soil burial test

5.16.6 Preservation of textile materials by anti-microbial modification

Anti-microbial modification of textile materials can be obtained by:

- addition of the microbicidal agent to the spinning mass of (semi-)synthetic fibers;
- application of the microbicidal agent to the textile material that is fixed to the textile material by chemical reaction;
- application of the microbicidal agent to the textile material that is fixed to the textile material by a polymeric or resin-forming additive.

At the design stage of the textile products with anti-microbial finishes or modifications, it is important to take into consideration how the product will be prepared and to what stresses and strains it will be subjected, in addition to the function of the textile. Chemical, thermal and mechanical effects may impair its biological effectiveness.

Anti-microbial modifications based on addition of the active agents to the spinning mass and based on application to the textile by chemical reaction, can provide anti-microbial effects that are permanent to washing and chemical cleaning.

Two types of anti-microbial modifications can be distinguished:

- microbicidal modification aims to actively kill micro-organisms;
- microbistatic modification aims to limit the growth of micro-organisms.

In order to obtain the strong biocidal effect needed to avoid mould formation on the textile materials and to avoid decomposition of the textile materials, it is needed that active component diffuses out of the textile material to the micro-organism (e.g. by water through hydrolytic decomposition or dissolving). Various classes of biocidal agents that can provide this effect, are:

- *metallic compounds* The oldest biocidal agents are metallic compounds. In general, complexing metallic compounds cause an inhibition of the active enzyme centres, i.e. they inhibit metabolism. This is also referred to as oligo-dynamic action or effect, i.e. it is effective in minute quantities. The effect is greatest with cadmium and reduces gradually with silver, brass, copper and mercury. With gold, platinum, iron, aluminium and zinc, the effect does not occur at all. Cadmium compounds have been used, but their use has decreased sharply due to its toxicity (see 'Legislation', 'Voluntary Safety- and Environmental Labels' and chapter 4). Various silver compounds are very popular. Several practical applications are known where fine zeolite particles loaded with silver- (and other) ions are added to the spinning bath of acrylic-, polyester- and polyamide fibres (commercial examples are "Kanebo Lifefresh-N" and "Kanebo Bactekiller", the latter also marketed by Acordis as "Diolen Bactekiller"). Silver is significantly more effective against Gram-negative than Gram-positive bacteria and fungi. It is also 3-4 times more effective at pH 8 than pH 6.

Textile materials dyed with many metal-complex dyes containing copper in deep shades also show biocidal effects.

Copper naphthenate (II, 8.1.12a.) is used, but only for outdoor applications due to its objectionable odor.

- *chlorinated phenolic compounds* It started with the disinfection of wound-dressings with phenol (II, 7.1.). Later, chlorinated phenols (II, 7.5.) like pentachlorophenol and 2,4,6-trichlorophenol have found wide use for protection cellulosic outdoor textile materials from mould formation and rot. Due to their toxicity, the use of these products is greatly reduced (see 'Legislation' and chapter 4). Other phenolic microbicides are o-phenylphenol, p-chloro-m-cresol (II, 7.3.1.) and 5,5'-dichloro-2,2'-dihydroxydiphenyl-methane (II, 7.7.3.). In general these compounds are insoluble or poorly soluble in water. Only at very high pH (pH 11,3 for o-phenylphenol) the compounds can be transformed into their water soluble salts. Since 1991 there is also a reliable technology to convert the products into a stable aqueous solution (Bayer, 1991). Examples of fibers with phenolics added to the spinning mass were Microsafe AM from Hoechst Celanese and Salus PP-fiber from Filament Fiber Technology (both U.S.A.).

A special microbicidal treatment based on chlorine is described (Williams, 1999) where cotton is grafted with N-halamines (II, 21.). This compound slowly decomposes releasing chlorine, which is the key to the compounds' effectiveness. The treatment can be easily refreshed by a dilute rinse of chlorine bleach and water (as is common to many industrial laundry processes).

- *organostannic compounds* (II, 19.5.-19.6.7.) Since the 70's, organostannic compounds have been used worldwide as active biocidal substances for the protection of materials. In general these substances are called tributylstannic (TBT). The most customary representatives of this group are:
 - tributylstannic oxide (TBTO);
 - tributylstannic benzoate (TBTB);
 - tributylstannic naphthanate (TBTN);
 - tributylstannic acetate (TBTA).

The compounds are highly toxic especially for aquatic organisms. They mainly have been used in anti-fouling paints for ships where it prevents algae, mussels and snails from sticking to the hulls (which would strongly impair the gliding capacity of the ships). Occasional applications to textiles are reported, mainly as preservative during transport. Due to their toxicity, the use of these products has been greatly reduced (see 'Legislation' and chapter 5).

- *formaldehyde-releasing compounds* The application of resins with slowly release formaldehyde has been greatly reduced due to the toxic and allergenic effects of formaldehyde (see 'Legislation', 'Voluntary safety- and environmental labels' and chapter 4).
- *antibiotic compounds* (II, 20.11.) Antibiotics are also classified as antimicrobial substances.
- *other compounds* Other compounds include natural terpenes, oxidising agents (including halogens and peroxo compounds), coagulants (predominantly alcohols), and radical formers (including halogens, isothiazolinones and peroxo-compounds).

However, when developing modern textiles for the outdoor sector, sports and leisure, the aimed effect is to prevent the transfer and spread of pathogenic micro-organisms and the deodorising or odor control effect. The objective of preventing the build-up of odors on textiles heralded a new era in the development of antimicrobial active principles. The deodorising effect consists of preventing the microbial decomposition of perspiration on the textile and thus preventing the release of odorous substances. This implies that the textile is worn where perspiration is formed, in other words, directly against the skin and that the micro-organisms are not necessarily destroyed, but that their metabolic properties to decompose perspiration are inhibited. But what is the effect of wearing a textile with an intense deodorising finish against the skin? This could damage the bacterial flora of the skin or the skin itself, or even pose a risk to the health of the person wearing the material. For these reasons biostatic finishes and modifications with discreet effects which cannot be washed out were developed for use e.g. in work wear and the food industry.

Another important challenge for which new microbistatic compounds are needed, is to limit the increase of dust mite populations and the exposure risks associated with the presence of dust mites and their allergenic elements. Millions of people suffer from allergies, skin irritations, asthma, or other respiratory diseases. The three major sources of indoor allergens associated with sensitization and subsequent allergic disease are dust mites, pets, and molds. Studies in different populations have shown that up to 85% of people with allergenic asthma, but only 5–30% of the non-asthmatic population are allergy prick test positive to mites. Mites feed on desquamated human skin. These skin cells are too dry for the dust mites to digest so they need to be broken down into a digestible food, which is facilitated by the fungus *Aspergillus repens*. By eliminating the *Aspergillus repens*, and therefore, the substance that dust mites feed on, one can control the increase of dust mite population.

Examples of compounds marketed in these fields of application are:

- 2,4,4'-trichloro-2'-hydroxy-diphenyl ether (II, 7.6.1.), Triclosan, and 4,4'-dichloro-2-hydroxydiphenyl ether (II, 7.6.2.), Tinosan which are marketed by Ciba, are used for the antimicrobial treatment of fabrics, e.g. incorporation into fibers such as acrylic, polyester, polyamide fibers.
- quaternary ammonium compounds (II, 18.) like biguanides, amines and glucoprotamines. These agents bind to microbe cell membranes and disrupt the lipopolysaccharide structures eventually resulting in the penetration of the membrane and breakdown of the cell. Typical examples of products used in the textile industry are:
 - Chitosan, an amino sugar based polysaccharide which is prepared by deacetylation and partial depolymerization of the naturally occurring chitin, and marketed by, amongst others, Heppe. Publications are made by Heppe (Heppe, 2002) and Knittel (Knittel, 2002).
 - Biguanides (II, 18.3.). A polyhexamethylene biguanide is marketed by Avecia in the product Reputex. Several studies on the effects of this compound are published by Yang (Yang et al., 2000) and Huang (Huang et al., 2000).
 - 3-trimethoxy-silyl-propyl-dimethyl-octadecyl-ammoniumchloride (II, 18.1.10.) is marketed by Aegis (USA) and Devan (Belgium, Europe). Several publications are made by Langerock on finishing (Langerock, 2002) and on modified fibers (Langerock, 2002).
- other compounds, like Polyethylene Glycols (Vigo et al., 1999) whose microbicidal effect is attributed to the surfactant-like properties which disrupts cell membranes due to their dual hydrophilic-hydrophobic characteristics.

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5.17 Industrial wood protection

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5.17.1 Foreword

This chapter aims to outline the use of microbicides in wood protection formulations, which are applied by industrial pre-treatment processes. This includes a description of wood structure, the influence of wood degrading organisms, methods of determining wood preservative performance and the types of microbicides and formulations used to control the degradative process in different end uses.

The wood protection industry is changing rapidly at the present time due to the increased pressures on the use of microbicides generally. As a result, the role of a wood protection formulation is changing with greater emphasis being placed on aesthetic properties as well as the selection and design of end-use specific products. In this way, the industry is gradually moving away from the situation where a single product is able to treat all commodities such as the copper chrome arsenic (CCA) type product. In achieving this goal, the specific properties of individual microbicides are being thoroughly researched and formulation chemistry is playing a much more important role than in the past.

5.17.2 Wood as a raw material

Wood remains one of the most versatile, durable and aesthetically pleasing substrates of all building materials. Whilst there have been trends in the growth and utilization of alternative substrates such as UPVC, wood continues to find favour in many applications including both structural and decorative end uses.

In addition to the obvious beneficial characteristics of wood such as its excellent strength properties, workability and aesthetic appeal, wood will always be highly regarded for its position as a 'renewable resource'. However, this term is misleading in that there is a clear distinction to be made between the highly durable hardwood such as teak, and the non-durable plantation species of both softwood such as spruce, pine and fir, and hardwoods such as beech and birch. Indeed there is increasing awareness and attention focussed towards the destruction of the tropical hardwood resource, and the resulting ecological damage that inevitably results from this. In contrast, the use of managed forest systems allows continued supply to the building industry of this most valuable of materials and at the same time minimising the impact on the environment. In order to meet these requirements however, the efficiency of use of wood and wood products must be maximised. To this end, it is essential to overcome the inherent biodegradable nature of the non-durable species. This can be achieved through the controlled application of wood-preservative systems, which contain biocides that specifically target the groups of organisms able to degrade this substrate. There still remains a significant lack of understanding as to the improvements that can be made to the durability of many timber species by the application of these preservative systems.

This chapter aims to summarise this subject area, focussing on the most commonly encountered types of wood degrade, their recognition and the range of wood preservative formulations used to prevent degrade.

5.17.2.1 *Wood structure and chemistry and its importance in wood protection*

At the simplest level, wood as a raw material has been described as 'a series of interconnecting holes surrounded by food'. This casual observation, whilst accurate from the viewpoint of the wood decay fungus or termite, inadequately describes the depth of research carried out on wood structure. A general, but modern overview of wood structure, is provided by Fengel and Wegener (1989). This text details the chemical structure of wood and the chemistry of the major cell wall components, cellulose, hemi-cellulose and lignin as well as the extractive components. A broader and visually based overview of wood structure is provided by Meylan and Butterfield (1972). This text allows the reader to gain insight into the three-dimensional nature of wood. A third text that provides additional information describes the flow characteristics of fluids into wood structure and the factors affecting them (Siau, 1971). Wood structure and anatomy varies significantly according to both genus and species. For the purpose of this section, wood used by the construction industry can be categorised into hardwoods (broad-leaved species) and softwoods (coniferous species). However, once converted into a dimensioned substrate at the sawmill, differences become more difficult to distinguish. Identification will often need expert guidance and involve both visual (anatomical) as well as chemical differences. At the simplest level, both hardwoods and softwoods

consist of two distinct and functional groups of elements termed 'heartwood' and 'sapwood'. The sapwood is located within the outer portion of the tree and consists of living cells that, in the main, act as conductive and storage tissues. In contrast, the inner heartwood zone is comprised of cells into which so-called 'extractive' compounds are liberated. These compounds typically darken this portion of the timber but also add durability through their bioactive nature. Many hardwoods are typified by larger zones of heartwood showing increased properties of durability. A measure of this durability provides the architect or builder with the ability to select the most appropriate timber, or combination of timber and preservative treatment. In this way, the construction industry can consider the use of a wood preservative formulation as a means of increasing durability of a given timber to achieve a level of protection equal to, or even greater than that provided by the most durable of hardwood species. The durability of commercially available timber is described in the European Standard EN 350 (1994), parts 1 and 2. This durability refers exclusively to the heartwood portion of the timber.

In addition to durability, there are a number of physical and chemical properties which directly affect the application of wood preservative products. Perhaps the most important of these is the permeability. The permeability of a given timber species to preservative penetration is closely related to the anatomical differences in wood structure. This can be considered on two distinct levels, the macro-distribution and the micro-distribution of preservative. Macro-distribution is dependant on the flow through the gross wood structure, whilst micro-distribution is dependant upon the preservative penetration into individual cell types and cell walls.

As with all wood properties, the permeability and therefore flow characteristics of the preservative are anisotropic; that is, dependant upon the direction and therefore cell type through which the fluid is penetrating. Generally, axial tissues provide the highest flow rates followed by radial and then tangential permeability. Axial penetration utilizes the tracheids of softwoods and vessels of hardwood species as the main conducting pathways. Between individual cell elements however, the fluid must negotiate the pit membrane structures. In hardwood species, vessel elements most often have un-occluded pit membranes (sieve plates) and often form open tubes through which high flow rates can be achieved. In contrast, flow of preservative in softwoods differs considerably having complex bordered pit membranes between adjacent tracheids. This makes axial penetration significantly more tortuous in many softwood species. Exceptions exist in both hardwood and softwood species with formation of tyloses forming interruption in the flow path in hardwood vessels, and the aspiration of bordered pit membranes in softwoods such as spruce providing reduced flow characteristics.

Radial and tangential penetration of preservatives is a more complex process involving a wide range of cell types. Radial penetration involves both the ray parenchyma and ray tracheid tissues (Siau, 1971). The permeability of the ray tissues is highly species specific however, and yet many commercial treatments must rely on this flow path for the majority of preservative uptake. This results from the way in which timber is sawn to provide maximum benefit of the wood strength properties, with only a small percentage of available wood surface providing an axial surface. As an added complication, the heartwood of most timber species is much lower in permeability. This holds true for both softwood and hardwood species alike. A classification for preservative penetration and retention is given in European Standard EN 351-1 (1996).

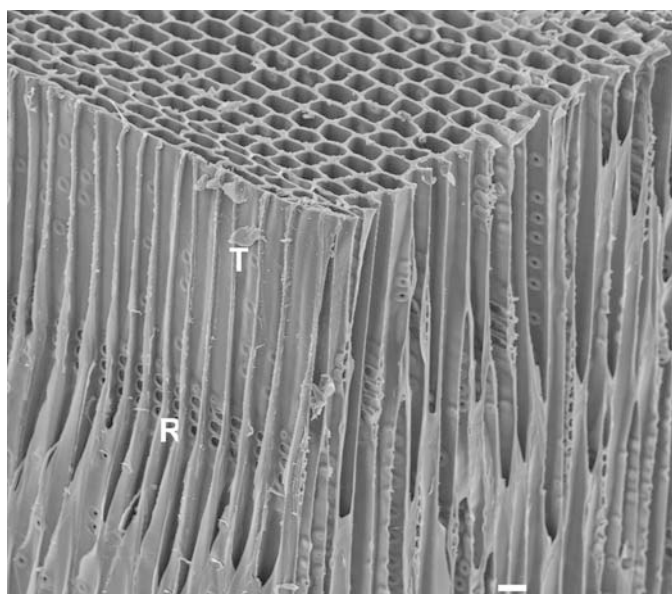


Plate 1 Photomicrograph of pine showing axial tracheids (T) and ray tissues (R) Bar = 100 μ m Reproduced with permission, Mike Hale.

5.17.3 Agencies of deterioration and their occurrence

Wood as a construction material is susceptible to biological degradation in almost all end-use situations. This results from the wide range of microorganisms that are able to utilise wood as a food source including bacteria, actinomycetes, fungi and insects including termites. The susceptibility to degrade by these organisms varies according to the environment in which the timber is used. Most generally, where timbers remain dry in service, the biodegradation is limited to beetle or termite attack. Where timber is exposed to the environment, or remains wet or in soil contact, the biodegradation rate can be much greater due to the wider range of organisms able to utilise the substrate under these conditions. This, in turn, requires a higher performance from the wood preservative formulation. In order to provide a convenient way of describing the requirements for the wood preservatives, a description that is receiving increasing acceptance has been developed based on a hazard-class system. Whilst there is not a global consensus of opinion on the system, the most popular is based upon a five (5)-class system as described in European Standard EN 335-1 (1992). This is summarised in Table 1 below.

A similar system based upon use-category is also described in the American Wood Preservers' Association Standard (2002). In addition, consideration is being given to a Hazard Class 6 specifically for the application of formulations to freshly felled timber (anti-sapstain treatments). Table 1 provides clear insight into the properties that a wood preservative formulation must possess in order to be effective.

5.17.3.1 *Degrade of wood by insects*

In all 'hazard classes', insects, including termites can cause significant degrade. The ability of insects to degrade wood begins in the living tree and significant economic loss results from infestation by insects. Indeed, reports on attack of timber in service are often confused with damage that occurred to the living tree or freshly felled log. A concise overview of the role of insects in wood decay is provided by Eaton and Hale (1993). The authors present a global picture of the occurrence of the various Orders of insects that are predominant on dry wood. In selecting the most commercially significant insects, the Orders Coleoptera (beetles) and Isoptera (termites) are undoubtedly the most important.

5.17.3.1.1 *Degrade of wood by beetles.* The beetles include 3 families, Anobiidae, Bostrychidae and Cerambycidae and all can be significant in the degrade of wood structures. Visual identification of beetle attack can be either by identification of the adult or more commonly by the characteristic end patterns of damage caused to the wood structure. This damage results from the activity of the larval stage and thereafter emergence resulting in a 'flight' or exit hole. In addition, the wood residue or 'frass' left in the tunnel has a characteristic shape and size and this is often used as an aid to the identification of the type of attack. Furthermore, attack by the beetles can be either timber species specific, or timber type specific. For example, attack of timber by *Lyctus brunneus* (powder post beetle) will be restricted to the sapwood of wide-pored hardwoods such as oak. In contrast, the common furniture beetle (*Anobium punctatum*) is able to degrade a wide range of hardwood and softwood species.

5.17.3.1.2 *Degrade of wood by termites.* Termites are the most destructive of all insects and from a global perspective, the most destructive of all organisms to wood components. With reference to wood protection, termites are classified as belonging to 3 distinct groups; the drywood termites, dampwood termites and the subterranean termites. The distinction between these refers to the ability of drywood termites to attack wood that is above ground i.e. out of soil contact. In contrast, dampwood and subterranean termites require a source of moisture and are therefore only able to attack timber in soil contact. There are six families within the Order Isoptera; the Termitidae, Rhinotermitidae and Mastotermitidae belong to the subterranean group, the Termopsidae to the dampwood group and the Kalotermitidae and Hadotermitidae belonging to the drywood group. Whilst all termites can cause significant wood degrade, it is the subterranean group that are probably the most economically significant. Most recently, the spread of the Formosan termite (*Coptotermes formosanus*) in the southern USA has raised the profile of termite attack and this has resulted in an increase in the research effort for means

Table 1 European hazard class system

Hazard class	Service conditions	Typical uses	Biological agents
1	Interior, dry	Framing, roof timbers	Beetles and Termites (Insects)
2	Interior, damp	Framing, roof timbers	Insects and Decay Fungi
3A	Protected exterior	Exterior joinery	Insects, decay fungi and disfiguring (bluestain) fungi
3B	Unprotected exterior	Deck boards	
4A	In-ground	Fence posts	As HC-3 plus soft rot fungi
4B	Fresh Water	Cooling tower	
5	Marine	Piles	As HC-4 plus marine borers



Plate 2 House longhorn adult beetle (*Hylotrupes bajulus*).

to control the extreme destruction this insect can cause in building structures. Termites are social insects living as a colony with individuals performing specific functions of foraging, defence, colony repair or reproduction. These 'castes' can be taxonomically distinguished although in the context of wood protection, it is the foraging 'worker' termites that are responsible for the major proportion of the structural damage and these are therefore the target for the wood protection formulation. The occurrence of insect attack is geographically related. This is particularly true for termites whose colonial environment means that the life cycle is completed outside of the building. Although there are exceptions as to the occurrence of termites, they are restricted in their activity based on minimum temperatures. The hazard to termite attack is therefore reduced according to the distance from the equator, and absent at extremes of latitude.

5.17.3.2 Bacterial degrade of wood

The significance of bacteria in the process of wood deterioration is receiving increasing attention. The focus is on two areas of research; understanding the degradative capacity and mechanism of bacterial degrade of wood, and also the roles of bacteria in the succession patterns of wood degrade. This latter area continues to grow in importance as the industry sees the move from heavy metal-based wood preservative formulations to metal-free, organic-based wood preservative systems. A distinction should be made at this stage between wood inhabiting bacteria and wood degrading bacteria. Whilst wood-inhabiting bacteria may indeed have a wood degradative capacity, they may also be important as a result of their ability to cause biotransformation of active ingredients or other components of wood preservative formulations (Williams, 1990). The bacteria, including the filamentous types (Actinomycetales) are considered to be primary colonisers of wood in many instances, particularly where the moisture content is elevated. Marine, freshwater and soil contact environments (Hazard Classes 4 and 5) are the most obvious situations, but any timber component that becomes wet in service for sufficient periods of time may include bacteria as a significant part of the population. This can include timber structures above ground such as decking as well as coated material such as window joinery and cladding (Hazard Class 3). In these lower hazard classes, bacteria may cause limited degrade of the wood structure, but their importance to the degrade of active ingredients has been recorded (Warburton and Hughes, 2002). This also holds true for freshly felled timber subject to mould and sapstain degrade, and the role and significance of bacteria to the performance of antisapstain products has, until recently, been underestimated (Williams, 1990; Cook, Sullivan and Dickinson, 2002). In soil and water contact where elevated moisture contents are present for longer periods, the degradative capacity of bacteria becomes equally important. Research into this area has been inhibited by the inability to isolate, into pure culture, the causal organisms. However, several distinct decay morphologies are now recognised including tunnelling bacteria, cavitation bacteria and erosion bacteria (Nilsson and Daniel, 1983; Singh, Nilsson

and Daniel, 1987; Singh et al., 1994). In contrast to fungal and insect attack, decay by bacteria is slow. However, it should be recognised that the requirements for performance of many wooden structures extends to decades, particularly in many of the higher hazard environments. In this context, bacterial decay as well as bacterial transformation of active ingredients plays a most significant role in wood degrade, and thereafter, in the performance of preservative treated wood.

5.17.3.3 Fungal degrade of wood

Fungal degrade of wood is most readily described by the end result of colonisation and enzymatic activity. Most simplistically, two types of degrade can be considered, visual degrade caused by mould fungi and staining fungi, and structural degrade caused by wood decay fungi. Most generally, mould and stain results from the growth of microfungi belonging to the ascomycotina, zygomycotina and the deuteromycotina within, or on the wood surface. Wood decay can also include the deuteromycotina, but in addition, involves the basidiomycotina.

5.17.3.3.1 Visual degrade by mould and staining fungi. Significant discoloration by mould and staining fungi can occur on both freshly converted timber and commodities in service. The occurrence of mould is ubiquitous in these situations although a distinction is commonly made between sapstain fungi, growing exclusively on the freshly felled substrate and bluestain fungi, occurring on timber in service. Both the species of fungi causing defacement, and the preservative active ingredients and methods of application vary according to the end use.

Sapstain and mould, as the term implies, occurs on freshly felled timber. The discoloration is generally limited to the surface of the timber and is visible as a result of the development of large numbers of coloured spores (conidia). They are commonly shades of green for example; *Trichoderma* spp., but black and brown moulds such as *Aspergillus* spp. and *Penicillium* spp. are also common. In contrast, sapstain fungi are able to invade the whole of the sapwood region of both logs and sawn timber. Perhaps the most characteristic form of degrade can be seen in recently felled logs in which a wedge-shaped pattern of staining may be observed.

The staining results from the gradual pigmentation of the hyphae within the wood cells. The progress of staining can be very rapid with some species showing typical hyphal elongation rates of up to 5.0 mm per day. In tropical climates, even faster colonisation rates have been observed, with *Botryodiplodia* spp. demonstrating colonization rates of up to 10.0 mm per day on timber species such as *Hevea brasiliensis* (rubberwood). Typical sapstain fungi include genera such as *Ophiostoma*, *Ceratocystis* and *Leptographium* (Williams, 1987).

In contrast, bluestain in service, whilst sharing similar visual characteristics, results from the growth of microfungi on wood that becomes wet only occasionally. It has been observed that this occurrence is part of the normal weathering procedure of wood surfaces. Typical bluestain fungi, such as *Aureobasidium pullulans*, have been shown to have the capacity to utilize the degradation compounds resulting from the delignification of the wood surface (Schoeman and Dickinson, 1998). This type of stain is also commonly seen under clear coatings, or as emerging sporophores through poorly protected opaque coatings. Whilst these fungi are most well known for their capacity to reduce the economic value of wood through visual defacement, they have also been observed to penetrate cell walls using fine penetration hyphae. This type of cell wall penetration is also

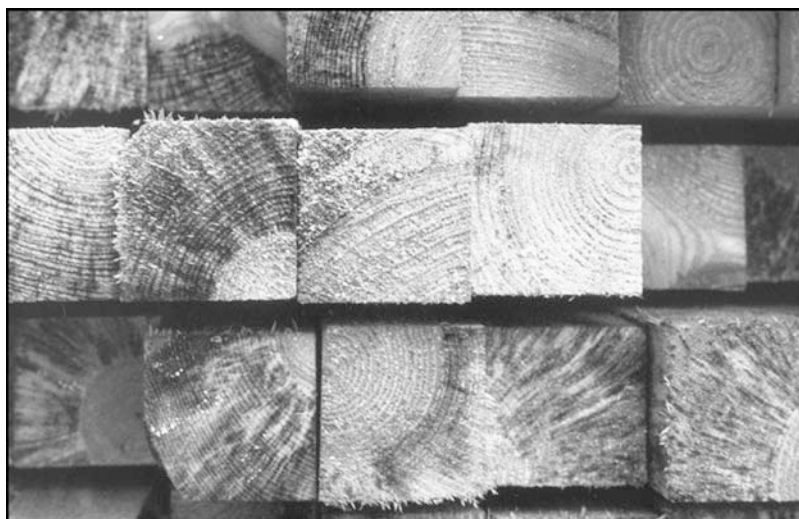


Plate 3 Sapstain on pine showing typical colonization pattern of ray tissues.

characteristic of the soft-rot fungi and indicates therefore, the capacity of some species in this group to resort to more significant wood degrade should the environmental conditions become suitable.

Most recently, mould fungi have received increased attention and media coverage due to the association with the so-called 'sick building syndrome'. Although there is still a poor understanding and a tenuous link between the occurrence of illness and the presence of specific mould fungi, it is also clear that the presence of spores and release of volatile components (mycotoxins) by certain mould fungi can result in significant human health issues.

5.17.3.3.2 Wood decay fungi. Significant wood decay by fungi can be caused by colonisation of ascomycotina, deuteromycotina and basidiomycotina. The ascomycotina and deuteromycotina are associated with degrade in wet environments including freshwater and soil contact. Basidiomycete degrade of wood also occurs extensively in soil contact but will occur in any situation given adequate wood moisture content and includes the brown rot and white rot fungi. In general, white rot fungi favour degrade of many hardwood species in wetter environments. A good overview of the categories of wood decay is described by Zabel and Morrell (1992).

Soft rot fungi. Soft rot degrade of wood is typified by degrade of the secondary cell wall and utilisation of carbohydrate material almost exclusively. As the term implies, surface softening is a typical symptom of attack but this type of degrade is most commonly identified by the specific pattern of cell wall decay caused by these fungi. Most typically, two types of cell degrade can be observed; cavity attack resulting from the enzymatic degrade of the secondary cell wall, and erosion attack resulting from degrade surrounding the hyphae on the cell lumen surface (Hale and Eaton, 1986; Leightley and Eaton, 1977). Cavity attack provides highly distinctive visual patterns of degrade, the most common of which are diamond-shaped or diamond-ended elongated cavities in the S₂ layer of the secondary cell wall. Typically these follow the microfibrillar angle of the wood cell wall. The presence of diamond cavities within the secondary cell wall viewed in longitudinal section and using polarised light microscopy is the most common means of identifying this kind of attack. There are several key fungal species associated with soft rot attack including *Chaetomium globosum*, *Phialophora hoffmanii* (*Lecythophora hoffmanii*), *Monodictys* spp. and *Hemicola allopallonea*. However, many of the early colonising microfungi that are initially scavengers, do have the ability to resort to soft rot activity depending upon environmental conditions.

The soft rot fungi have special significance with respect to the presence of microbicides and wood preservative formulations. They are known to be highly tolerant to a wide range of active ingredients and may thereby perform an important role in primary colonisation and detoxification of preservative treated wood. A good example of this is the susceptibility of hardwoods such as eucalyptus species, to soft rot attack even following treatment with highly effective wood preservatives such as CCA. Research indicated that this was due to the very high copper tolerance of these organisms, exacerbated by poor preservative distribution, particularly in the difficult to treat fibre bundles of these wood species (Greaves, 1972).

Brown rot fungi. Brown rot decay of wood is typified by a dark, reddish brown appearance of the wood structure and a characteristic cubical checking. This appearance results from the degradation of carbohydrates

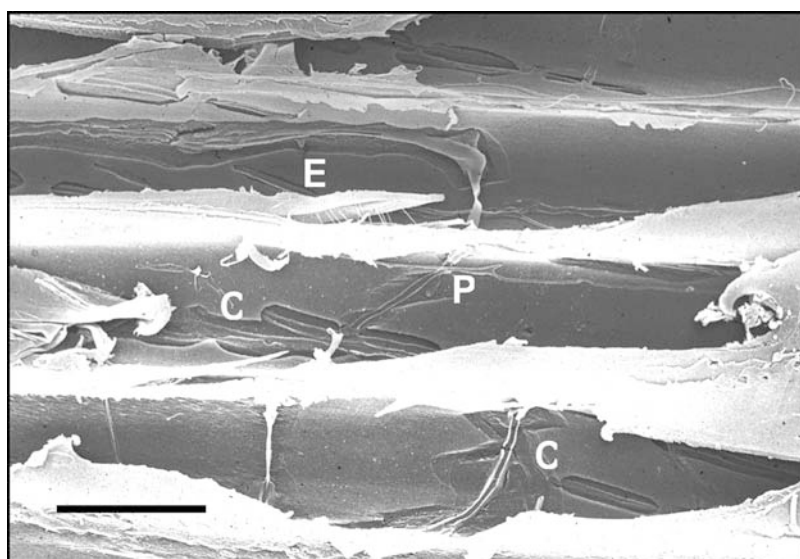


Plate 4 Soft rot attack of *Alstonia* spp. showing typical cavity attack (C), cell wall erosion (E) and cell wall penetration (P). Bar = 50 µm. Reproduced with permission, Mike Hale.

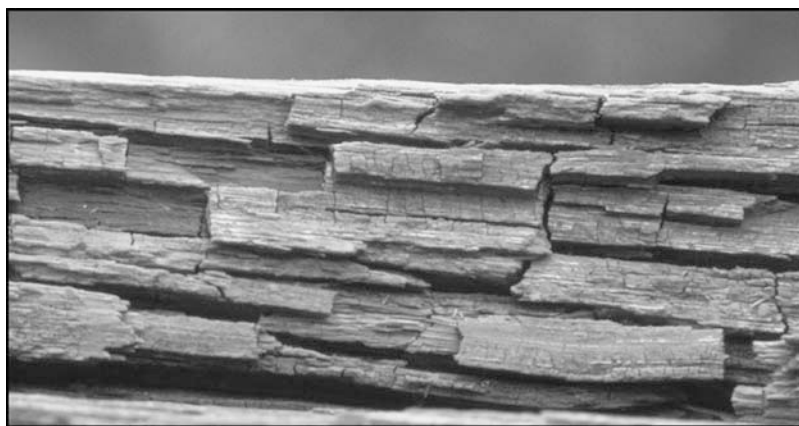


Plate 5 Brown rot in pine showing typical cubical checking. Reproduced with permission, Mike Hale.

leaving a high proportion of lignin residue. Invasion of the wood structure by brown rot fungi via axial tissues is rapidly followed by the formation of distinct boreholes in the cell wall. Thereafter a gradual dissolution of cell walls can occur at some distance from the fungal hyphae resulting from a highly diffusible enzyme system. There are great number of brown rot fungi that are able to degrade wood although only a few of these are used for the evaluation of wood preservative formulations. These are limited to fungi commonly associated with the decay of construction materials, fencing and utilities. The following are most commonly recorded; *Coniophora puteana*, *Serpula lacrymans*, *Antrodia viallantii*, *Oligoporus placenta*, *Gloeophyllum trabeum*, *Gloeophyllum sepiarium* and *Lentinus lepideus*. *Serpula lacrymans* differs somewhat from others within this group and is described as a dry rot fungus. This results from its ability to degrade wood remote from an immediate source of moisture. The brown rot fungi also vary significantly in their tolerance to wood preservatives. As an example, *Oligoporus placenta* and *Fibroporia viallantii* are known as copper tolerant brown rot fungi. This is reflected in their ability to tolerate high levels of copper-based wood preservatives. They are able to achieve this via a series of metabolic 'solutions'. This includes secretion of oxalic acid to form insoluble copper oxalates, sequestering with mucilage surrounding the fungal hyphae and formation within the fungal cell of copper-protein complexes or copper sulphate/copper polyphosphate compounds. The inclusion of arsenic and to some extent chromium in CCA preservatives significantly improves the performance of copper as a wood preservative by inhibiting the growth of these fungi. More recently this tolerance has led to the development of specific wood preservative formulations containing additional active ingredients such as the azoles; tebuconazole, propiconazole and cyproconazole and quaternary ammonium compounds such as benzylkonium chloride and didecyldimethyl ammonium chloride. A review of copper tolerant brown rot fungi and their significance in preservative treated wood is given by Williams and Fox (1994).

White rot fungi. White rot fungi are often described as either simultaneous white rots or sequential white rots as a result of the order in which they are able to degrade both the cellulose, hemicelluloses and lignin portions of the wood structure. The process of delignification results in a distinct whitening of the wood, hence the name. Associated with this effect and distinct from the brown rot fungi is the fibrous pattern of decay resulting from degradation of the compound middle lamella and separation of individual wood cells. The micromorphology of attack is also distinct from the brown rot type decay in that the white rot fungi do not possess a diffusible enzyme system. The degradation of the cell wall therefore tends to progress from the cell lumen surface through the S_3 , S_2 and S_1 layers of the secondary cell wall finally degrading the primary wall and middle lamella, resulting in fibre separation. The most commercially significant white rot fungi include *Trametes versicolor*, *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. *T. versicolor* is known for its high decay capacity in hardwood species such as beech and birch and testing methods such as the European Standard EN 113, often utilise this organism for evaluation of preservative performance in hardwood species. *P. ostreatus* is best known for its occurrence in wood composite material such as chipboards and plywood. White rot fungi, whilst showing very low tolerance to heavy metals such as copper and zinc, are tolerant of a wide range of other organic fungicides, including many azoles (tebuconazole and propiconazole) and quaternary ammonium compounds.

5.17.3.4 Marine borer damage to wood

Timber exposed in the marine environment provides the opportunity for the widest range of wood degrading organisms including bacteria, fungi, beetles and marine borers. Degradation by marine borers is of course limited to that portion of the structure which is either inter-tidal or submerged. There are two distinct groups of borers belonging to the molluscs and crustaceans. The molluscs include the shipworms (teredinidae) and the pholads

(pholadidae). The most common of these are the shipworms with a wider climatic occurrence although pholads are more tolerant of brackish water (Eaton and Hale, 1993). Attack of wood by the shipworm is typified by the formation of a calcareous lining to the tunnel bored into the wood. Destruction of the timber in this way can be rapid and extensive with tunnels extending up to two metres in length. The fact that the shipworm utilizes the wood components as its food source is significant in the selection of wood preservative formulations to prevent this type of attack. Of the pholads, *Martesia* spp. are probably the most economically significant. The pholads differ from the teredinidae through the relatively short tunnels they produce (often no greater than 40 mm) which have no calcareous lining.

Of the marine crustaceans there are three economically significant groups; the limnoridae, the sphaeromatidae and the cheluridae. Most well known of the limnoridae is *Limnoria* (the Gribble), which causes superficial attack of the wood surface. However, repeated attacks can result in severe damage to pilings over an extended period of time. In contrast, attack of wood by the sphaeromatidae (of which *sphaeroma* is the most important) can result in rapid and severe degrade resulting from the larger (up to 5 mm) holes bored perpendicular to the wood grain. The cheluridae, including common species of *Chelura* seem to be associated with attack by *Limnoria*.

5.17.4 Methods of assessment for wood preservatives

Determination of the performance of biocides and wood preservative formulations is a most complex subject. All methods, whether laboratory or field yield highly variable results stemming not only from variations in natural durability of the wood substrate, but also because of the different performance requirements according to both hazard class and geographical location. As with most industrial applications, testing can be divided into laboratory evaluations and field evaluation. However, for wood preservatives, it is the extended performance requirements (greater than 50 years in some structures) that present specific difficulties in determining the likely in-service performance of a candidate formulation. To this end, laboratory evaluation tends to be relied upon only for screening purposes or to determine the suitability for use in a given environment (spectrum of activity). In addition, laboratory evaluations in many countries are used for approval purposes using regional standards and codes of practice.

Field assessment methods aim to create a more realistic in-service type environment for wood preservatives. However, it is often forgotten that even these tests represent an accelerated environment utilizing smaller timber sections and aggressive exposure conditions.

Most recently, wood preservative companies together with various professional institutions and universities have increased the effort to understand the link between laboratory, field and in-service performance. This has become particularly important with the introduction of biodegradable organic biocides. Focus is now being placed on monitoring the depletion rate of active ingredients as a measure of ultimate performance rather than relying solely on the onset of microbiological deterioration. Such methodology is, however, in its infancy at the present time.

The European Standard EN 599-1 (1996) provides a good description of testing requirements according to hazard class. Similar descriptions may be found which are used on other continents, for example, the American Wood Preservers Association Standards (AWPA). Brief descriptions of test types are given below:

5.17.4.1 Stain and mould fungi

5.17.4.1.1 Sapstain and mould. Although no standardised tests are available for mould and sapstain fungi it is accepted that field-testing yields the most reliable results. These trials are carried out during spring and autumn periods when environmental conditions are optimum for growth of the fungi involved. The most common methodology involves briefly dipping (up to 20 seconds) freshly sawn samples of wood in a solution of the chemical under test. Emphasis is placed on using timber that has been very recently felled and is free from pre-colonisation by sapstain and mould fungi. Dependant upon the timber species, heartwood may be eliminated from the test as far as is practicable. Treated samples (typically 1–2 metres × 2.5 cm × 10 cm) are then close stacked or 'sticker stacked' (using spacers) and left for a period of at least 10 to 12 weeks.

Assessment of performance is by a visual grading system although individual types of defacement may be recorded (stain, mould or decay).

5.17.4.1.2 Bluestain in service. Bluestain in service can be evaluated either in the laboratory or as part of a field evaluation using coated timber samples such as L-joints (EN 330, 1993). In the laboratory, accelerated results can be obtained although treated samples are often pre-conditioned by external weathering or by a period of exposure to UV in the laboratory (EN 152, 1988). Performance is evaluated by determining the degree of visual defacement.

5.17.4.2 Laboratory fungal decay testing

5.17.4.2.1 Basidiomycete fungi. The performance of a wood preservative formulation against a range of individual wood decaying basidiomycetes can be determined using a simple wood block exposure method.

Typical methods include EN 113 (1996) and AWP A E10-01 (AWPA, 2002). These tests and similar procedures use small samples (typically 8–12 g in mass), which are impregnated with test solutions of the preservative product using a simple vacuum impregnation process. The samples may also be subjected to accelerated ageing procedures, for example water leaching according to EN 84 (1997). These procedures determine to some degree, the relative permanence of the active ingredients in the wood although it is now realized that in the absence of organisms that may bio-transform the preservative components, only a limited determination of longevity can be achieved. Treated samples are exposed over a nutrient agar or soil surface (dependant upon regional standards) on which the test organism has been cultured. Aseptic conditions are maintained for the duration of the test (12 to 16 weeks) and weight loss of the sample is used as a measure of preservative efficacy. It is accepted that a mean mass loss of less than about 3% would represent an effective treatment. Evaluation of the degree of attack in this way allows the derivation of a set of values known as the 'toxic limit values' or 'threshold values'. These describe a range for the level of preservative in the wood that is effective in preventing fungal degrade. The data may also be depicted graphically as a plot showing mass loss against preservative retention in the wood samples. These tests can be used for a variety of both white and brown rot basidiomycetes as well as alternative timber species.

5.17.4.2.2 Soft-rot fungi. Due to the specific conditions of exposure needed for optimum soft rot degrade, non-sterile soil tests have become accepted as the most reliable medium for evaluation of products to control soft rot in wood (see European Standard ENV 807, 2001). These tests utilize small treated wood samples exposed in a compost-type soil. High moisture content favours soft rot (and bacterial) degrade and typically moisture levels of 90 to 110% of the water holding capacity of the soil are used. No specific fungi are added to the soil and development of natural micro flora is sufficient to result in a reliable test so long as the period of exposure is sufficient (up to 36 weeks). Due to differences in susceptibility to decay, a more rapid evaluation can be obtained using hardwood species such as *Betula* spp. (birch) or *Fagus* spp. (beech). In these tests it is important to use a preservative standard; for example, copper chrome, to determine the relative aggressiveness of the individual test. As with the basidiomycete tests however, the results derived from this test are difficult to interpret since performance ultimately relies upon the 'reservoir' of active ingredient within the wood sample. Higher levels of residual preservative in the sample will inevitably give superior test results and performance is therefore related closely to the depletion rate of active ingredients during the test exposure period.

5.17.4.3 Insect testing in the laboratory

Evaluation of the performance of both beetles and termites can be carried out in the laboratory.

5.17.4.3.1 Testing against beetles. Tests against beetles include methods to determine the preventative efficacy of wood preservatives as well as eradication action. In addition, the methodologies are confused by the requirement for testing either superficially applied formulations or fully impregnated samples. In summary, these tests aim to evaluate either the ability of larvae to survive the treatment process, or for adults to successfully lay eggs on the wood substrate. Brief descriptions of the relevant European Standard tests, as examples, are given below:

(a) *Anobium punctatum*, the common furniture beetle. There are four tests described within the European Standards for the evaluation of performance against *Anobium punctatum*; EN 48, (1998); EN 49-1, (1992), EN 49-2, (1992) and EN 370 (1993). EN 48 aims to simulate the situation in which wood that is already infested with the larvae of *Anobium punctatum* may attack a newly treated section. To simulate this, larvae are placed in pre-drilled holes of Scots pine samples and their ability to degrade the wood is measured by weight loss. EN 370 determines the eradication action of a preservative against larvae of *Anobium punctatum* by placing larvae in proximity to a treated veneer placed onto the wood surface. This test determines the ability of *Anobium* to emerge through the treated zone of already infested material. EN 49 parts 1 and 2 utilize more of the life cycle including egg-laying and larval survival on the wood surface. These tests aim to determine the level of preservative that prevents hatching and survival of the larvae. Part 1 of this standard evaluates surface applications, and part 2 evaluates impregnated products.

(b) *Lyctus brunneus*, the powder post beetle. Tests to evaluate the effectiveness of preservatives against *Lyctus brunneus* include EN 20-1 (1992), (superficial application), EN-20-2, (1993), (impregnated samples) and ENV 1390, (1994) (eradication action) and are carried out on European oak samples. For the evaluation of pre-treated timber, EN 20-2 exposes treated samples to live adult beetles and determines an effective level of preservative that prevents larval development.

(c) *Hylotrupes bajulus*, the house longhorn beetle. Evaluation of preservative performance against the house longhorn beetle is the most common test carried out since it represents the one of the most significant wood degrading insects in Europe. Three European tests are used; EN 22 (1974), EN 46 (1988) and EN 47 (1988). EN 22 determines the eradication action of preservatives against *Hylotrupes* spp. The methodology is similar to the *Anobium punctatum* test (EN 48) with larvae introduced into pre-drilled holes in treated Scots pine blocks. EN 46 determines the effectiveness of surface applied preservative by placing larvae into a narrow gap between a glass plate and the treated wood surface of a small pine block. Efficacy is determined by assessing the level of larval mortality and the presence or absence of tunneling. In contrast, EN 47 is used to evaluate fully treated samples. Once again larvae are introduced and mortality and ability to tunnel are used as criteria for effectiveness.

5.17.4.3.2 Determination of effectiveness against termites. Determination of preservative efficiency against termites in the laboratory is limited in its value compared with other insect tests. This holds true due to the wider range of environmental conditions in which termites may attack wood. Beetles are most commonly a problem in the degrade of wood inside buildings, (Hazard Class 1 and 2). In contrast, termites are able to degrade timber in all environments with the exception of commodities in direct contact with fresh water (HC-4) or in the marine environment (HC-5). However, portions of such components not in contact with the aquatic environment. Two European laboratory test methods are available; EN 117 (1989) and EN 118 (1990). Similar methodologies are used in the USA (AWPA, 2000), Australasia and Japan. The EN 117 method evaluates the performance against termites for penetrating treatment processes whilst EN 118 is used for superficial application treatments such as dipping. These tests use visual examination, mass loss and termite mortality as the criteria by which performance of the preservative is evaluated.

5.17.4.4 Field evaluation methodologies

For the evaluation of the wood preservatives in Hazard Class 3 or higher (above ground, ground contact, fresh water or marine) there can be no real alternatives to field evaluations. However, there is a distinction to be made between in service trials that use full-size commodities and components, and field evaluations. The difference here is that trials use artificially small samples (e.g. stakes instead of posts) and more often than not an exposure site with an artificially high degree of hazard. For example, in soil stake tests, soils are chosen for their known decay capacity rather than their geographical location. Altering the parameters in this way allows an 'acceleration' of degrade such that comparative performance to known standards can be determined.

5.17.4.4.1 Above ground (HC-3) testing. A number of field test protocols have been developed to evaluate wood preservative formulations. For fungal decay above ground, two tests are favoured; for coated (painted) wood samples that simulate window joinery (millwork), an L-joint type test is most commonly used (for example, EN 330, 1993). This is a mortise and tenon jointed sample arranged in an L configuration. The wood is impregnated with preservative, assembled and then coated. Prior to exposure, the joint is re-broken to break the paint film. This effectively accelerates the decay process. For non-coated use (e.g. decking) a 'lap-joint' test is preferred. This is similar to the L-joint but uses a rather flatter profile sample to provide a larger joint surface area. Samples for this test can be envelope treated or fully impregnated with preservative. Assembled samples are placed horizontally on an appropriate rack. Performance in both test types is by visual examination of the wood samples but particularly the joint area, which is most susceptible to decay. In Europe, the lap-joint test is being developed as a European Standard (DD ENV 12037, 1996) and in the USA as AWP standard E16-98 (AWPA, 2002). In addition, tests are being developed in the USA for evaluation of above ground performance against termites. These tests involve placing small sample blocks in proximity to an area with an active termite population (or colony) and allowing access via feeder (non-treated) strips to the test samples (ground proximity test).

5.17.4.4.2 Ground contact (HC-4) testing. In order to simulate the performance of treated wood in soil contact, numerous field exposure methods have been developed which involve small 'stakes' exposed for about 50% of their length in a field test site. Sites are chosen for their high biological activity for either termites and/or wood decay organisms and stakes are evaluated on an annual basis. As for above ground evaluation, a visual index of decay is used for assessment. Most commonly, a diligent wood preservative manufacturer will test the efficacy of a new product in multiple locations in order to determine the effect of both climate and soil type on preservative performance. Test standards include the European standard EN 252 (1989), and the AWP standard E7-01 (AWPA, 2002). In order to obtain consistent and meaningful data on the potential long-term performance of a wood preservative, such tests may be carried out over extended periods. However, this is a controversial topic and it is becoming recognized that it is extremely difficult to determine the service-life of a treated commodity solely by the evaluation of such tests. In addition, efforts to develop test methods that are



Plate 6 L-joint testing (EN 330) for coated and uncoated samples.

intermediate between laboratory and field have been explored. These tests, known as 'fungal cellar' or 'accelerated field simulator', are becoming more popular and have used smaller dimension material exposed in various soils under optimum climatic conditions (Jermer, Bergman, and Nilsson, 1993).

5.17.4.4.3 Evaluation of preservatives in the marine environment. Determination of the performance of wood preservative products in the marine environment may include an evaluation of both decay and insect attack for those portions of the commodity not in contact with seawater. In order to determine the suitability of a product in preventing degrade by marine borers however, there is no alternative to a field evaluation. Such tests often involve the pressure impregnation of the product into small samples (ca. 200×75 mm), which are then connected together with rope in the form of a 'ladder'. The 'ladder' is weighted and then exposed in an appropriate location of the intertidal range to optimize the colonization by Gribble and Shipworm, or Pholads.



Plate 7 Forest Research Institute field stake test site, NZ.

Preservative evaluation standards include European Standard, EN 275 (1992), or American Wood Preservers Association standard, E5-00. (AWPA, 2002).

Determination of performance is through visual examination of the degrade caused by the borers, and the period of evaluation can be anywhere from 3 months to 5 years depending on the water temperature and occurrence of borers at a given location. A concise review of both the occurrence of marine borer attack and testing procedures is given by Eaton and Hale, 1993.

5.17.5 Wood preservative formulations

Wood preservative compositions can be most easily distinguished based on either the presence of heavy metals in the formulation, such as copper, zinc and tin, or their absence in metal-free (organic) formulations. This applies equally to both fungicide and insecticide based compositions. Many authors also consider Tar Oil preservatives such as creosote as a separate class of compounds. However, for consistency, these are discussed under organic wood preservatives in the following sections.

Many metal-containing and metal-free wood preservative systems can be applied in either organic solvent based or water based systems. Water based wood preservatives utilize either water soluble compounds, such as borates, or emulsified (low water solubility) active ingredients. In contrast, organic solvent systems can use a range of solvent types from low volatility heavy oils to lighter fraction solvents such as the white spirit types. Whilst there is an increasing legislative move away from volatile organic solvents in Europe (VOC directive), these systems are still used for applications by brushing, dipping, spraying or double vacuum treatments. Most commonly, the organic solvent preservative systems are preferred by the higher quality joinery (millwork) manufacturers for a number of reasons including improved dimensional stability (distortion and grain-raising), rapid surface drying and compatibility with coating systems. However, there is increasing emphasis on high quality water-based wood preservatives as an alternative to this traditional method for treating joinery components. Heavily oil based preservative systems are targeted more towards heavy-duty industrial applications, such as the treatment of transmission poles, where the general public are less likely to come into direct contact with the treated article.

5.17.5.1 Industrial application of wood preservative formulations

There are a wide variety of processes available for the application of preservatives to wood products. These are generally divided into two areas based on the 'result' of treatment; superficial application processes and penetrating processes. In order to distinguish between these, penetrating processes are defined as those methods, which are designed to overcome the natural resistance to preservative uptake. Superficial processes are generally used where the requirement for preservative penetration is limited, i.e. in the lower hazard classes, HC-1 and HC-2. Dipping is the most common process used and packs of timber are immersed in the treating solution for periods ranging from 20 minutes to a few hours. This method is also used for the commercial application of antisapstain compounds.

Penetrating processes are traditionally divided into the so-called 'double vacuum' treatments, and 'vacuum-pressure' treatments. Both processes take place in enclosed systems that include a treatment vessel and wood preservative storage tank. With a double vacuum treatment system, the wood is placed in the treatment vessel and air removed under vacuum. The treatment vessel is then flooded with preservative that is absorbed by the wood. Following treatment, the preservative is pumped back to storage and a final vacuum applied to remove excess preservative fluid from the wood surface. The vacuum pressure processes employ similar technology, but include a period of pressure that allows much greater depths of penetration to be achieved. Whilst the description presented here is somewhat simplistic, these processes have evolved considerably to allow accurate preservative uptake and active ingredient retentions using modern microprocessor control equipment. Such processes are also highly regarded for the effective reduction in worker exposure to the treatment chemicals compared with the open tank dipping-type operations.

5.17.5.2 Wood preservative design

Regardless of the type of active ingredient used, a wood preservative system must be designed to meet a series of requirements. These include permanence in the wood structure (from water leaching, evaporative ageing, biological degradation or chemical degradation), spectrum of activity, overall performance, handling characteristics, health, safety and environmental characteristics and of course, cost. These requirements are dependant upon the specific end use of the treated article. For example, a treated roof truss must be protected from insect attack and resistant to evaporative loss of the preservative. In contrast, a fence post may be required to withstand all types of fungal decay and termite attack and also be resistant to water leaching and evaporative loss. In order to achieve

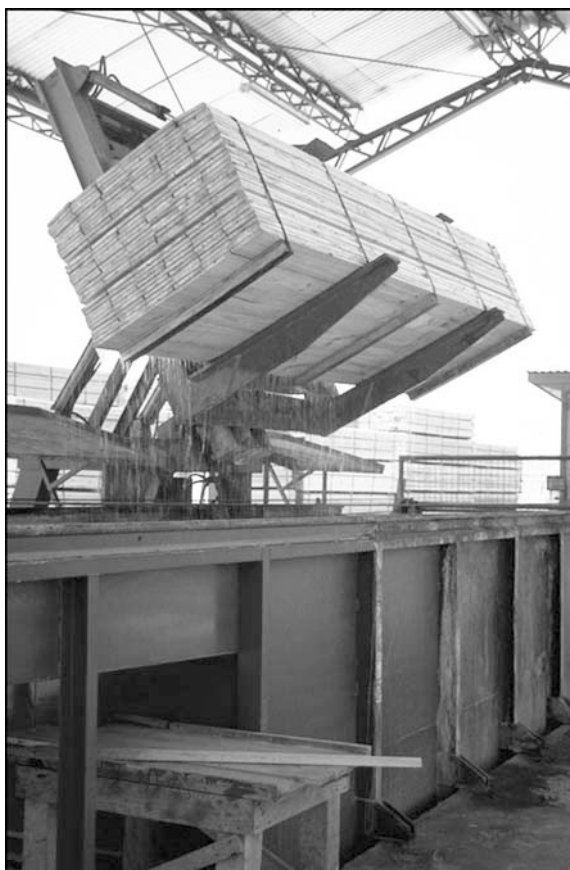


Plate 8 Dipping treatment plant for antisapstain products.

these goals, the wood preservative system will need to be designed accordingly and, whilst some wood preservative systems may be effective in all of these end uses (for example, copper-chrome-arsenate) other systems may be more limited in their applicability (for example, simple boron salts). In addition, there is a move within some parts of the global wood preservative industry towards greater utilization of metal-free wood protection systems. Use of these wholly organic preservative systems has already dominated the treatment of internal building timbers where the hazards to biological degrade is reduced. However, the replacement of metal-containing preservative in more hazardous end uses has proven more difficult. This has resulted from the complexity in designing wood preservative systems, using inherently 'biodegradable' organic biocides derived from the agricultural and pharmaceutical industries, that will have sufficient permanence in the wood structure to give the long periods of protection required by the industry. However, increased knowledge of formulation chemistry combined with



Plate 9 Vacuum-pressure impregnation treatment plant.

better understanding of the importance of spectrum of activity, is gradually providing solutions to this difficult problem (Warburton and Hughes, 2002; Foster et al., 2002).

5.17.5.3 *Metal-based wood preservative systems*

Both inorganic as well as organo-metallic compounds [II, 19.]* have been extensively used in wood preservation. Most commonly products based on arsenic, chromium, copper, tin and zinc have been included.

5.17.5.3.1 *Zinc-based wood preservatives.* Some of the earliest wood preservatives used simple zinc salts such as zinc chloride. Permanence in the wood of these compounds is of course low in a wet environment and performance therefore limited. Further development using chromium to improve zinc permanence led to the development of treatment standards, for example within the AWP. These products however, demonstrated poor performance against some of the heavy metal tolerant fungi and are no longer used or indeed specified. Zinc however, is still used and specified by the AWP in the form of an ammoniacal-copper-zinc-arsenate formulation. Zinc has also been used extensively in light organic solvent treatments in the form of naphthenates [II, 8.1.12], or the so-called zinc 'soaps'. These are based on the use of branched chain carboxylic acids such as isononanoic acid and neodecanoic acid. The use of the carboxylic acid provides additional spectrum of activity against the metal-tolerant basidiomycetes such as *Postia placenta*. Zinc soaps have been used in organic solvent-based systems as preservatives for the protection of internal construction products and joinery components with the gradual move towards water-based wood protection products as well as the development of metal-free systems, the industry has seen a significant reduction in the use of these compounds.

5.17.5.3.2 *Copper-based wood preservatives.* Without doubt, copper-based wood preservatives form the principal and most effective group of wood preservative compounds used by the industry. In comparison with zinc, copper provides approximately twice the level of activity on an equivalent weight basis. The spectrum of activity is broad and copper-based formulations can be shown to be effective against most fungi, including moulds and stainers, brown rot, white rot and soft rot species, as well as insects including termites. However, tolerance to copper is characteristic of a number of organisms. The two most significant groups which impact on the wood preservative industry resulting from this tolerance are a number of fungi imperfecti (moulds) and the copper tolerant brown rot fungi such as *Postia placenta*. Within the range of copper-based wood preservatives the most successful formulation will also contain additional active ingredients (secondary actives) to ensure adequate protection against these organisms (Williams and Fox, 1994).

Copper-based preservatives can be formulated as organo-metallic, wholly organic, or mixtures of organic biocides and copper compounds. The copper organo-metallic systems include compounds such as copper naphthenate [II, 8.1.12a] and copper-8-quinolinolate [II, 13.3a.]. Copper naphthenate is still specified for use in the USA by the AWP, but only as an organic solvent-based preservative treatment. Water-based emulsion treatments have been developed but have been shown to be less effective. Most commonly, these compounds are applied in a heavy 'fuel' type oil and used for industrial heavy-duty applications. Copper-8-quinolinolate (Cu-8) is also specified as a wood preservative by the AWP in organic solvent systems but has also been formulated for use in water-based antisapstain treatments. The most accepted formulation of Cu-8 has been the acid solubilized system which, whilst giving excellent temporary protection to freshly felled timber, is now used less frequently due mainly to instability in use and the corrosive (to mild steel) nature of the solution.

Inorganic formulations of copper have formed the mainstay of the wood preservative industry for many years. Known most commonly as the copper salts, the most successful formulations have been based on copper plus chromium to give an insoluble copper chromate effectively fixed within the wood. Discussions still ensue on the detailed mechanism of copper fixation from these systems including the reactivity with cell wall constituents. Suffice it to say within the current context, that the copper remains very well 'fixed' within the wood and is highly resistant to water leaching in service. The issues over copper tolerant fungi were noted early on in the development of these wood preservative systems as well as the limitations in the control of termites. For this reason, research by Kamesan in 1933 resulted in a patent for a mixture of copper, chromium and arsenic, most commonly referred to today as CCA. As perhaps the most successful wood preservative and certainly the most widely used, CCA has become the standard by which many more recent innovations are measured. This success as a wood preservative results from the exceptional combination of spectrum of activity, permanence in the wood during service and low cost. CCA has been used for internal building timber using a reduced amount of active ingredient as well as in heavy-duty applications such as poles, sleepers (cross-ties) and marine applications. Even in high-risk soil contact applications, service lives of commodities in excess of 50 years have been recorded.

Variations on these chemistries have also been developed including ammoniacal copper arsenate and ammoniacal copper-zinc arsenate, copper chrome boron and copper chrome phosphate. These alternatives have

*see Part Two – Microbicide Data

been designed for specific applications. For example, the ammoniacal systems provide improved penetration of difficult to treat (less permeable) species of timber. However, none have been as universally accepted as CCA. Most recently however, there has been a move away from these types of wood preservatives on a global scale focussed mainly on the concerns over both chromium and arsenic as components of these formulations. This has resulted in the development of alternative systems which maintain copper as the primary active ingredient. A wide range of alternative water-based systems have been evaluated which combine copper with conventional organic fungicides in most cases. These include copper-azole [II, 14] (CuAz), copper citrate (CC), copper dimethyl dithiocarbamate [II, 11.10] (CDDC), copper-n-cyclohexyldiazonium dioxide [II, 20.1b.] (Cu-HDO) and alkaline copper quaternary ammonium compounds [II, 18.1.] (ACQ). At the time of writing, the most widely used alternatives on a global basis are copper-azole, copper HDO and copper-quaternary products. Typical specifications for these preservatives can be found in the AWWPA standards (2002). The move away from chromium has of course required development of alternative fixation mechanisms for copper. Most of the new alternatives rely on the formation of a copper-complex using either ammoniacal, amine or polyamine compounds [II, 18.2.]. This provides a water-based wood preservative system into which secondary biocides such as quats are solubilized (such as ACQ) or emulsified such as the azoles in copper-azole formulations. To date, these alternative wood preservatives are providing excellent performance in many end-uses.

5.17.5.3.3 Tin-based wood preservatives. Tin has also been used as a wood preservative for many years. Traditionally organo-tins based upon a tri-alkyl group such as tri-n-butyl tin oxide [II, 19.5.] (TnBTO) were most commonly used. These were formulated in organic solvent systems with the focus on above-ground applications, particularly joinery treatments. TnBTO, whilst globally accepted for its wood preservative efficacy is generally being replaced by more environmentally acceptable alternative fungicides such as azoles. The main drive in this change has been the move by both regional authorities as well as the International Marine Organization (IMO) in restricting the use of TnBTO in anti-fouling paints applied to ship hulls.

5.17.5.4 Organic wood preservative chemicals

Organic wood preservatives, whilst misleading in description, refer to the group of metal-free fungicides and insecticides that have been derived from the agricultural and pharmaceutical industries. A wide range of chemistries have been evaluated and used in practice, although there is a clear drive towards the utilisation of active ingredients with reduced environmental persistence, improved toxicity profiles and improved specificity. Fungicides such as pentachlorophenol (PCP), and other halogenated phenols as well as chlorinated hydrocarbons such as lindane (gamma-hexachlorocyclohexane) and cyclodiens such as aldrin and dieldrin, have gradually either been removed from use, or restricted due to toxicity or environmental unacceptability. In evaluating alternative chemistries however, perhaps the most limiting factor in their success has been the rapid biodegradation of these active ingredients in service. Whilst this factor provides a favourable environmental profile, the wood preservation industry has had to carry out extensive research in order to find suitable alternatives. Perhaps the best example of this to date is the difficulty the industry has encountered in finding an alternative to PCP [II, 7.5.4.] for use in protecting freshly felled timber (antisapstain treatments). This results not only from the excellent spectrum of activity of the molecule, but also its biological persistence and therefore resistance to microbial (bacterial) degradation. Arising from this research, a number of chemistries have emerged which have been demonstrated to provide good protection to treated wood in service. This technology is advancing quickly and allows the extension in use of metal-free (organic only) wood preservative systems into more hazard classes and uses, particularly HC-3 (above-ground) and HC-4 (below-ground). Compared with many water-based, metal-containing formulations, metal-free preservatives require the addition of insecticides as appropriate for the end use. The most common active ingredients and their end uses are summarized below.

5.17.5.4.1 Boron. Boron as a semi-metallic compound requires separate consideration in its use as a wood preservative. Whilst used as a component in many wood preservative formulations (e.g. copper-chrome-boron), it has also been used extensively as a stand-alone treatment for wooden components. Boron has advantages and disadvantages as a wood preservative. Whilst it is not susceptible to degrade by fungi and bacteria in wood, this also means that it is persistent in the environment. When used as borate [II, 8.2.1./9.11.], it has good water solubility but this contributes to lack of permanence in wood in wet environments. As a leachable component therefore, borate tends to be restricted to internal, protected end uses where it acts as a fungicide and also insecticide, including termites. Borate is limited in its spectrum of activity however, and whilst efficacious against higher fungi (basidiomycetes) is limited its activity towards deuteromycetes and ascomycetes (moulds and stains). An additional benefit noted at high retention of borate treatment is its property as a fire retardant. This combination of properties has led researchers towards significant investigation of mechanisms by which to fix or 'immobilize' boron in the substrate. Success to date however, has been limited due mainly to the counter effect of reduced bioavailability and thereby efficacy. Combination with metals such as zinc has also been extensively used and these borates have lower solubility and therefore improved permanence. Targeted mainly in treating

wood-composite materials such as oriented strand board (OSB), zinc borate is currently introduced into the board during the manufacturing process and used for internal construction panels, especially where protection from termites is required.

5.17.5.4.2 Triazole fungicides [II, 14.]. Azoles currently hold a dominant position as organic fungicides used in wood preservation. The earliest azole investigated was azaconazole [II, 14.3.] and formulations were focussed towards the control of microfungi, particularly in antisapstain formulations. The value of azoles as wood preservatives was quickly recognized with tebuconazole [II, 14.1.] and propiconazole [II, 14.2.] demonstrating further improvements in performance. These remain the dominant azoles used in wood protection formulations today. Further developmental work has demonstrated the improved activity of azoles combined in mixtures. Both tebuconazole and propiconazole provide very good control of brown rot fungi, but require higher active ingredient retentions to control white rot. Similarly, their spectrum of activity is poor towards the microfungi and for applications where surface degrade by mould or stain is common (HC-3 exposure), then additional fungicides such as IPBC or dichlofluanid may be used. As with all metal-free wood preservatives, insecticides are commonly added to complete the spectrum of activity. Additional azoles have also been investigated as wood preservatives including cyproconazole [II, 14.4.], hexaconazole and myclobutanil. Azoles also currently form one of the key formulation components that are added to the new generation of copper-based alternatives to CCA; the copper-azole preservatives.

5.17.5.4.3 Alkyl ammonium compounds [II, 18.1.]. Alkyl ammonium compounds and particularly the quaternary ammonium compounds (quats) rival the triazole fungicides in their contribution towards the wood preservation industry. As water soluble active ingredients that readily adsorb to wood cell wall components, quats are used in a wide range of applications including antisapstain treatments, interior and exterior heavy duty wood protection. The spectrum of activity includes microfungi (stains, moulds and soft rot) and basidiomycetes, although the performance against white rot fungi is again somewhat reduced. Quats also have a degree of efficacy against insects increasing their value in many end use situations. The quats are highly biodegradable and whilst this is an environmental advantage, their degradation rate in wood in wet environments contributed to problems encountered when they were first used in New Zealand (Vinden and Butcher, 1991). Arising from this early research was the development of the use of quats in lower hazard environments (HC-1 and HC-2) and combination with other active ingredients, particularly copper. In the same way as azoles and copper have been combined to produce broad spectrum wood preservatives, quats and copper (as alkaline copper quat) have also been shown to be an effective combination. Two main types of quaternary ammonium compounds are used; alkylbenzyl dimethylammonium chloride (such as benzalkonium chloride, [II, 18.1.2.], BAC) and the dialkyl-dimethyl ammonium chlorides (such as didecyl-dimethyl ammonium chloride, [II, 18.1.4.] DDAC). Research work is continuing on this important group of active ingredients and focuses towards the investigation of alternative anions such as propionates or carbonates which eliminate the presence of the corrosive chloride ion.

5.17.5.4.4 Creosote as a Wood Preservative. More accurately described as coal-tar [II, 7.] creosote, this compound has been used extensively for heavy-duty wood protection of commodities such as fencing, transmission poles and railway sleepers (cross-ties) for many years. Creosote is effective against all types of wood degrading organisms including fungi, beetles, termites and marine borers. This activity results from the complex mixture of organic compounds included in a wood preservative formulation. A typical analytical breakdown of a creosote preservative has been described by Betts (1991). The main active ingredients are recognized as tar oils such as phenols and cresols, tar bases such as quinolines and neutral oils including naphthalene and phenanthrenes. Many of these compounds have significant vapour pressures that give rise to the characteristic odour associated with creosote treated wood. Creosote is specified for use in a number of countries and standards exist in both the USA (AWPA, 2002) and in Europe by the West European Institute for Wood Impregnation (WEI). Whilst the characteristic odour is a perceived benefit by some users, both the volatility and mobility of creosote in treated commodities such as poles gives rise to handling concerns. The problems of mobility or 'bleeding' have been thoroughly investigated and more recent research has attempted to address these issues through the development of water-based emulsified formulations such as pigment-emulsified creosote (PEC), (Greaves et al., 1985). Creosote remains a popular wood preservative due to the long service history of treated commodities. However, handling, health and safety and environmental concerns are resulting in restrictions on its use in a number of countries.

5.17.5.4.5 Isothiazolones [II, 15.]. Isothiazolone fungicides have been evaluated for a wide range of end uses in wood protection including mouldicides, antisapstain compounds and wood preservatives. Three types are commonly distinguished; the water soluble mixture of 2-methyl-4-isothiazolin-3-one (MIT) plus 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) [II, 15.3.], the non-chlorinated 2-n-octyl-4-isothiazolin-3-one [II, 15.4.] (OIT), and the dichlorinated 4,5-dichloro-2-(n-octyl)-4-isothiazolin-3-one [II, 15.5.] (DCOIT). The mixture of

MIT and OIT has been used extensively as an additive in wood protection solutions to prevent contamination by common mould fungi. Being water-soluble however, they have not proven to afford significant wood preservative performance. The usage rate is also limited in many countries due to the sensitization properties common to many isothiazolones. OIT has been evaluated for a number of end uses including mouldicides and antisapstain compounds as well as an additive to control stain on coated surfaces. The spectrum of activity however, is limited primarily due to the mould and staining fungi, although all of these isothiazolones show good bactericidal properties. DCOIT has been more thoroughly evaluated as a wood preservative compound, demonstrating good performance against a wide spectrum of wood decaying fungi as well as some degree of efficacy against certain beetles. To date however, whilst DCOIT is registered for use as an active ingredient in a number of countries, it is not commonly included in wood preservative formulations.

5.17.5.4.6 Iodine containing compounds. Iodine containing compounds are used broadly as antiseptics and fungicides in a wide range of applications. In wood protection, the most commonly used iodine-containing active ingredient is IPBC (3-iodo-2-propynylbutyl carbamate) [II, 11.1.]. IPBC is used for a wide range of applications with activity against the mould and staining fungi as well as basidiomycetes. However, its activity towards the former group of moulds and staining fungi has focussed the wood protection industries development towards its use as an antisapstain formulation and to control stain on treated joinery (millwork) components. For higher hazard end uses, including joinery and above ground wood protection, products are now available which combine the excellent activity of IPBC against the lower fungi, with the well-proven performance of the azoles to control the basidiomycete group of fungi. It is likely that these types of formulations will form the basis of the next generation of preservatives that are heavy metal free particularly in above ground use (HC-3).

5.17.5.4.7 Phenylsulphamide fungicides [II, 16.]. There are two phenylsulphamide preservatives that have been evaluated and used as components of wood preservative formulations; dichlofluanid [II, 16.5.] and tolylfluanid [II, 16.6.]. Both of these active ingredients target the mould and stain fungi, and have been used as components to control stain and mould in organic solvent-based systems, and in particular, for joinery (millwork) applications. However, they have very limited stability in water-based systems.

5.17.5.4.8 Aromatic fungicides. Pentachlorophenol [II, 7.5.4.] (PCP) and its water-soluble sodium salt (sodium pentachlorophenoxide) have been extensively used as wood preservative components. With a very broad spectrum of activity against most fungi, as well as insects, PCP has been successfully applied as its water-soluble salt for antisapstain treatments. Furthermore, its resistance to biotransformation has provided good long-term protection but has been a disadvantage in terms of its environmental acceptance. In addition, the presence of chlorinated dibenzo-p-dioxins as an impurity in many chlorinated phenols has caused concern due to the teratogenicity of such formulations. Pentachlorophenol has also been incorporated in many other wood preservative formulations and particularly in heavy oil-based preservatives for the treatment of commodities such as railway sleepers (cross-ties) and transmission poles. PCP is however, due to the issues noted above, becoming increasingly restricted in its use, with considerable research underway to find more acceptable alternative chemistries. Also included in the group of aromatic fungicides is chlorthalonil [II, 17.19.]. Chlorthalonil has low water solubility and is therefore formulated in either organic solvent or as water-based dispersion (Laks et al., 1997). As well as good performance against wood decay fungi, chlorthalonil is purported to deter certain termite species (Grace et al., 1992). However, it is more commonly combined with the insecticide chlorpyrifos, in order to complete the spectrum of activity. Chlorthalonil is also used as a component in antisapstain formulations, particularly in combination with carbendazim.

5.17.5.4.9 Benzimidazole fungicides. Of the benzimidazole fungicides, both thiabendazole [II, 15.9.] and carbendazim [II, 11.4.] have been evaluated as components of wood preservative formulations. Of these, carbendazim has been valued for its activity towards stain and mould fungi, particularly in combination with chlorthalonil as noted above. Carbendazim (a breakdown product of benomyl [II, 11.5.]) has very low solubility in most solvents and therefore is most commonly applied as a dispersion in antisapstain treatments. Carbendazim is however soluble at lower pH (as an acid salt), and has been used as a mould control agent in CCA treatment solutions. Acid salts of carbendazim (hydrochloride and phosphate) have been used in the treatment of Dutch elm disease.

5.17.5.4.10 Thiazole/benzothiazole fungicides. The thiazole fungicide TCMTB [II, 15.11.] (2-thiocyanomethylthio benzothiazole) was one of the earliest organic fungicides investigated for wood preservation. With low water solubility but good organic solvent solubility, it was evaluated for both antisapstain use and as a conventional wood preservative. It quickly became recognized for its good performance against staining fungi and was used as a component in many antisapstain formulations, often in combination with methylene-bis-

thiocyanate [II, 20.9.1.] (MBT). Research in this area however identified limitations in terms of its susceptibility to bacterial degrade and therefore rapid loss of activity during storage and transit of antisapstain treated wood (Williams, 1990).

5.17.5.4.11 *Miscellaneous compounds.*

MBT (methylene-bis-thiocyanate) [II, 20.9.1.]. As a water-soluble thiocyanate, MBT has limited use as a wood preservative although it has been extensively used in combination with TCMTB as an antisapstain compound. The use of this molecule in the field of antisapstain has been reviewed by Williams (1987), who found it to be highly diffusible when applied to the sapwood of recently felled timber. This diffusability has more recently been utilized in formulations to protect logs of radiata pine from sapstain development when exported from New Zealand (Chittenden et al. 2001).

Bethoxazin. Bethoxazin is a fungicide belonging to the group of compounds known as oxathiazines. Research has shown this active ingredient to have a broad spectrum of activity towards both bacidiomycetes and deuteromycetes, but with it's main activity towards microfungi belonging to the bluestain, sapstain and soft-rot group of wood degraders. This emphasises its importance to the wood protection industry being one of the few newer active ingredients with these properties. It also possesses activity towards a number of algae, providing additional benefits to the control of surface defacement on wood exposed to the environment. This molecule has been reviewed most recently by Forster et al. (2002).

5.17.5.5 *Insecticide components of wood protection formulations*

Whilst the heavy metal-based wood preservatives have insect protection capability, the organic and organ-metallic systems require the addition of a specific insecticide.

Most of the older generation of insecticides based on the chlorinated hydrocarbons (lindane, dieldrin, aldrin etc.) have now been removed from these end uses due to both toxicity and environmental concerns. However, the development of insecticides is a rapidly developing area of research with new generations of compounds demonstrating greater specificity, lower mammalian toxicity and reduced environmental burden.

The selection of the most appropriate insecticide is dependant upon a large number of factors including cost, stability in the preservative formulation, required rate of action and stability in the commodity to be protected. No finite recommendations can be made and ultimate selection will result from research into the performance and permanence of the insecticide in both laboratory and field tests. The most common groups of insecticides used in wood preservation today can be categorized as follows:

5.17.5.5.1 Pyrethroids. More accurately described as the synthetic pyrethroids, this group of compounds has been used extensively in wood preservation for control of both beetles and termites. Activity, cost and performance characteristics vary depending upon the specific compound but most readily accepted for use in wood preservative formulations are permethrin, cypermethrin, deltamethrin, bifenthrin and cyfluthrin. In terms of activity against wood boring beetles, cyfluthrin is estimated to be 20 times more effective than permethrin, 10 times as effective as cypermethrin and twice as effective as deltamethrin when compared in laboratory evaluative procedures. However, in practice other factors need to be considered, particularly the relative vapour pressures (and therefore evaporative loss) and, in wood in soil contact, the rate of biotransformation by colonising bacteria. The pyrethroids are effective as neurotoxins, and are axonic poisons.

5.17.5.5.2 Chloronicotinyl and neonicotinoids. Active as neurotoxins, this group includes the compounds imidacloprid, thiacloprid and thiamethoxam (Xamox). These compounds demonstrate a broad spectrum of activity including beetles and termites. These are very new chemistries for the wood protection industry although some products have already been registered in a few countries for lower hazard end uses. Both imidacloprid and thiacloprid are very specific in their activity towards insect nervous tissue as mimics of the neurotransmitter acetylcholine.

5.17.5.5.3 Pyroles and phenylpyrozoles. The pyroles such as chlorfenapyr act as metabolic toxins and work by uncoupling oxidative phosphorylation in the mitochondria. In contrast the phenylpyroazole, fipronil has a mode of action similar to the cyclodiens (e.g. aldrin) and acts as an axonic poison.

5.17.5.5.4 Insect growth regulators (IGR). IGR's are chemicals that are able to act on the endocrine and hormone systems of insects. Highly specific in their action, they have received much attention as potential insecticides for wood protection. Many of the newer IGR's mimic the juvenile hormone and effectively inform the insect to remain in the immature state. Adult insects treated with such products are also unable to moult

successfully and therefore cannot reproduce. Insecticides evaluated and used in wood protection formulations include fenoxycarb and flufenoxuron.

5.17.5.5.5 Chitin synthesis inhibitors (CSI's). CSI's are often included in the group of insect growth regulators although this is incorrect. The most established CSI's are the benzophenyl ureas including diflubenzuron, (Dimilin[®]) and hexaflumuron, (Sentricon[®]). As inhibitors of chitin synthesis, these chemicals prevent from making insects new cuticle thereby preventing them from moulting successfully to the next stage of development. The commercial product Sentricon, is being used extensively in termite bait protection systems in the USA.

5.17.5.5.6 Insecticides affecting water balance. The well-established active ingredient boric acid is included in this group of compounds. They are effective by disrupting the water balance within the insect, although boric acid is also thought to act as a stomach poison. Additional compounds that disrupt the waxy cuticle of the insect resulting in desiccation include silica aero-gels and diatomaceous earths, although these substances have not been used as wood preservative components.

5.17.6 Health, safety and the environment

The impact of health, safety and environmental controls has been significant to the wood preservation industry from a variety of standpoints. Superficially, it might be expected that the greatest impact would be from the restriction of specific actives or components, but it should be realized that the impact on the wood protection industry is far more significant than this, including manufacturing, distribution, use and disposal of the treated article.

From the global perspective, each country, each state or member state (in the European Community) may apply similar protocols for the regulation in use of the active substances used in a wood preservative formulation. The complexity of these requirements is certainly not a subject for this section, but it is clear that whilst the time frame for major change may be different on a global basis, the trends are the same. Put most simplistically, these changes have impacted on the wood protection industry in the following areas:

5.17.6.1 Restriction on the use of active ingredients

This is probably the most significant area of change for the industry. There have been restrictions and even removal from some markets of several compounds including the chlorinated hydrocarbon insecticides, such as DDT, Dieldrin and Lindane, organo tin compounds such as the tri-n-butyl tin compounds, chlorophenols, such as pentachlorophenol or its sodium salt, copper and zinc soaps and even creosote. This has resulted for a variety of reasons including high mammalian toxicity, carcinogenic, teratogenic or mutagenic concerns, irritation or sensitisation of individuals coming into contact with treated wood or product, or the impact on the environment. Recently, the most significant change within the industry has been the move away from chromium and arsenic based preservative formulations and in particular CCA. Within Europe, following the restriction in the use of CCA, the European Commission has published its intention to restrict the use of CCA treated playground equipment, sleepers (cross-ties), transmission and telecommunication poles and cooling tower timbers. In the USA, CCA has been under similar restrictions, but with few alternatives present in the market, this represents a dramatic change. At the beginning of 2001, 98% of all wood protection treatment in the USA was with CCA. However, in 2001, consumer and environmental groups began questioning the safety of the treatment related to its use in residential structures. This resulted in action by the industry and the Environmental Protection Agency (EPA) to raise the awareness of consumers as to the properties of the treated wood. However, early in 2002, the EPA announced a voluntary decision by the treatment industry to 'move consumer use of treated timber products away from a variety of pressure treated wood that contains arsenic, by December 31st 2003, in favour of new alternative wood preservatives'. This decision effectively prohibited the use of CCA treated timber for playground structures, decking, picnic tables, landscaping timbers, residential fencing, patio and walkways and boardwalks. In addition, the EPA strongly advised treaters to look for chrome and arsenic free alternatives to CCA preservatives. This action has resulted in an unexpected change to the industry with rapid growth of alternative formulations to CCA, based on organic biocides or mixtures of copper and organic biocides such as copper-azole, copper HDO and copper-quaternary ammonium mixtures.

5.17.6.2 Availability of active ingredients

Increasing environmental and health and safety legislation on a global basis has severely restricted the availability of new active ingredients. With a small critical mass, the wood protection industry can never command the requirement for the development and manufacture of new active ingredients destined specifically for this end use. Rather, the industry has spent considerable resource investigating the suitability of biocides sourced from the agricultural and pharmaceutical industries as potential new wood preservative compounds. This applies

to both fungicides and insecticides. With increasing requirement for data submission to the relevant authorities, as well as for compliance with the Biocidal Products Directive (BPD), the number of suitable active ingredients continues to diminish with time. It is interesting that this has probably led to the investigation of alternative methods of wood protection including wood modification and heat treatment. – See also Chapter 3 and Chapter 4.

5.17.6.3 *Effect on treatment process*

Health, safety and environmental factors have had a significant impact on the treatment process and particularly process control. Two examples serve well here; the move within the European industry from organic solvent based treatments for organic biocides to water-based treatments, and the very specific requirements for post-treatment handling of the treated wood. In the second example, it is the leaching of unfixed preservative as a result of rainfall on freshly treated material, and the resulting concerns over environmental impact which have driven the development of processes and practices such as steam fixation, or rapid drying of treated wood.

5.17.6.4 *Specification of wood preservatives*

There is a growing trend within the industry towards more stringent specifications of wood preservative treatment according to the performance requirements of the commodity in use. This is driven by both the cost of the treated article but also the understanding of the relationship between preservative retention and service life within a given hazard class. This particularly applies to the use of alternative treatment methods such as superficial application of biocides in place of the more conventional pressure impregnation process.

5.17.6.5 *Development of methods for the determination of environmental impact*

This is a rapidly developing area and involves close collaboration between industry and global regulatory bodies to derive methods which not only aim to accurately determine the loss to the environment of wood preservative components, but also the impact on susceptible organisms within the environment. The most recent developments are evaluating the potential for standard methods in which the use of treated wood in a specific end use can be modelled to determine the rate of flux of active ingredient to the environment. In Europe, this allows the determination of a predicted environmental concentration (pec) and this can be compared with predicted no-effect concentration for the active ingredients concerned (pnec). It is currently considered that a pec: pnec ratio of < 1 may determine the suitability of an active ingredient.

5.17.7 **Summary comments**

The science of wood protection is becoming increasingly complex from all aspects including product formulation, application, efficacy and health, safety and the environment. In addition, aesthetic properties such as colour, water repellency and long-term surface appearance are becoming as important to the consumer of treated wood products as wood preservative performance and service life. This in turn has led to a change within the wood protection industry with greater focus on the individual performance requirements for specific commodities and end uses. The selection of the most appropriate microbicides to meet these needs has become a critical part of the development process for new wood preservative formulations.

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Part Two - Microbicide data

Organisation of microbicide data

When trying to classify or to subclassify material protecting microbicides to their mode of action, e.g. as membrane-active and electrophilically active agents, it turned out that a clear assignment is not always possible. For that reason it has been resorted to chemistry's principle of classifying, i.e. organisation of microbicides by the following groupings:

Alcohols – Aldehydes – Aldehyde releasing compounds – Phenol derivatives – Acids – Acid esters – Amides – Carbamates – Dibenzamidines – Pyridine derivatives – Azoles – Heterocyclic N, S compounds – Compounds with activated halogen atoms – Surface-active agents – Organometallic compounds – Various compounds – Oxidizing agents

Such a classification provides in most cases the first necessary information about a microbicide's properties.

The data sets inform about the chemical and physical properties of the microbicides, their toxicity (including their ecotoxicity), their antimicrobial effectiveness and applicability. Sources for toxicity data are, if not otherwise indicated:

Registry of Toxic Effects of Chemical Substances (RTECS).

Lexicon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete (1989). Ed. H.P. Fiedler, Editio Cantor, Germany.

Product information and safety data sheets of microbicide suppliers.

All indications are given in good faith and conscience. This also applies for Occupational Exposure Limits, the Acceptable Daily Intake (ADI) Values, and to the data with regard to ecotoxicity and biodegradability of microbicides.

Supplier names do not claim to be always complete and up-to-date. Nowadays it is not rare that active agents have been discontinued, or being made by another company; corporate takeovers, or the shift of microbicides to a new company have also taken place.

Abbreviations

BPD	Biocidal Products Directive
CAS	Chemical Abstract System (or Service)
EEC	European Economic Community
E-No.	Substance-number in the EEC Directive for Food Additives
EEC-No.	Substance-number in the EEC Cosmetic Directive
EC-No.	Substance-number in EINECS
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New (Notified) Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Organisation
FIFRA	Federal Insecticide and Rodenticide Act (USA)
GRAS	Generally Recognized as Safe (USA)
K _a	acid dissociation constant
log	logarithm to the basis 10
MITI	Ministry of International Trade and Industry (Japan)
NCI	National Cancer Institute (USA)
No.	number
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
pK _a	negative logarithm of the acid dissociation constant
POW	octanol/water partition coefficient
TSCA	Toxic Substances Control Act (USA)
TSCATS	TSCA Test Submission
WHO	World Health Organisation

1. Alcohols

Alcohols are generally characterized as solvents possessing lipophilic properties as well as some degree of hydrophilicity due to the hydroxyl group. According to these properties they are more soluble in body fluids and take longer to eliminate than molecules devoid of hydrophilicity. However, alcohols are in general readily metabolised by oxidative processes (e.g. by alcoholdehydrogenase) leading to the corresponding aldehydes and acids, i.e. to compounds which are responsible for the typical toxic effects of alcohols. The defatting properties of alcohols are in line with their irritant action on the skin and respiratory system.

Microbicidal alcohols are, as a general rule, colourless volatile liquids. In numerous fields of application they thus offer the advantage of being effective without leaving residues.

Alcohols rank among the membrane-active antimicrobial agents; they are adsorbed at the cytoplasmic membrane which – as a multi-purpose instrument of the microbe cell – is particularly susceptible to functional disturbances. Membrane proteins form, for example, parts of enzymes that catalyse the transformation/decomposition of substances so that metabolites important for the cells' vegetation may form and pass the cellular membrane. The denaturation of membrane proteins by alcohols necessarily disturbs those processes and is the main reason for the alcohols' antimicrobial effect, which is limited to vegetative micro-organisms. Spores will not be destroyed. The virucidal efficacy is confined to a few viri. Alcohols are characterized by acting at a quick rate but only in comparatively high concentrations, preferably in the presence of water. Absolute ethanol for instance does have a dehydrating effect, but a lower denaturing effect than an aqueous ethanol solution. Gram-negative bacteria are more sensitive to alcohols than Gram-positive ones. By first approximation it can be said that the effectiveness of alcohols increases with their molecular weight. More complex is the mechanism of action of some unsaturated iodinated alcohols.

Whilst lower alcohols are chiefly employed to disinfecting hands and surfaces, higher alcohols lend themselves to use in preservatives – in cosmetics and pharmaceuticals rather than in industrial fluids.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.1. Ethanol
Chemical formula	C_2H_6O
Structural formula	CH_3-CH_2-OH
Molecular mass	46.07
CAS-No.	64-17-5
EC-No.	200-578-6
Synonym/common name	ethyl alcohol
Supplier	BP, HAYMAN, HULS, UNION CARBIDE
Chemical and physical properties	
Appearance	clear, colourless, burnable fluid
Boiling point/range °C (101 kPa)	78.2–80
Melting point °C	–114
Density g/ml (20°C)	0.79
Vapour pressure hPa (20°C)	58–59
Viscosity mPa · s (20°C)	1.2
Surface tension mN/m (20°C)	22.8
Refractive index nD (25°C)	1.3595
Flash point °C	12–13
Auto ignition temperature °C	365–425
Upper flammability limit %v/v i.air	15–19
Lower flammability limit %v/v i.air	3.3–3.5
Stability	volatile, hygroscopic
Solubility	complete in water, alcohols, acetone, ether, trichloromethane, benzene
Toxicity data	
LD ₅₀ oral	13700 mg/kg rat 5500 mg/kg guinea pig 9500 mg/kg rabbit
LD ₅₀ intravenous	4200 mg/kg rat 2300 mg/kg guinea pig
Irritant to skin	

Exposure limits	German/UK 1900 (1000) mg/m ³ (ppm)
	USA 1800 (1000) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

The presence of water is essential for the antimicrobial effectiveness of ethanol. Concentrations of 60–70% ethanol in water exhibit the strongest killing action (Price, 1950). Non-sporulating bacteria are rapidly killed by such concentrations, while ethanol at all concentrations does not affect bacterial spores (Russel, 1971).

Ethanol is mainly used in disinfectants for surfaces, instruments and for the skin, e.g. in hand disinfectants. Sometimes ethanol is applied as a preservative, e.g. in pharmaceutical and cosmetic products, where odourless and non-irritating preservatives are preferred. On the other side ethanol is the dominant solvent in cosmetics and toiletries, as it disposes of favourable properties such as consistency, purity and low toxicity.

Table 1 Minimum inhibition concentrations (MIC) of ethanol in nutrient agar

Test organism	MIC (%)
<i>Escherichia coli</i>	11
<i>Pseudomonas aeruginosa</i>	7.5
<i>Aspergillus niger</i>	6
<i>Rhizopus nigricans</i>	6

Table 2 The killing action (exposure time in seconds) of various concentrations of ethanol for various test organisms (Wallhäuber, 1984)

Test organism	Ethanol concentration (%)			
	60	70	80	95
<i>Staphylococcus aureus</i>	15	15	10	
<i>Staphylococcus epidermidis</i>	30	30		
<i>Streptococcus pyogenes</i>			90	
<i>Escherichia coli</i>	60	30	30	
<i>Serratia marcescens</i>		10		
<i>Salmonella typhosa</i>		10		
<i>Pseudomonas aeruginosa</i>		10		
<i>Mycobacterium tuberculosis</i>	60	30	30	30
Spores of <i>Trichophyton gyps.</i>			30 min	

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.2. 1-Propanol
Chemical formula	C ₃ H ₈ O
Structural formula	CH ₃ –CH ₂ –CH ₂ –OH
Molecular mass	60.10
CAS-No.	71-23-8
EC-No.	200-746-9
Synonym/common name	n-propyl alcohol, ethyl carbinol
Supplier	UNION CARBIDE, BASF

Chemical and physical properties

Appearance	clear, colourless, burnable fluid with a slight alcoholic odour
Content %	99
Boiling point/range °C (101 kPa)	96.5–97.5
Melting point °C	–126.2
Density g/ml (20°C)	0.8035
Vapour pressure hPa (20°C)	18.7
Viscosity mPa · s (20°C)	2.3
Refractive index nD (20°C)	1.385
Surface tension mN/m (20°C)	23.45

Flash point °C	23
Auto ignition temperature °C	405
Upper flammability limit %v/v i.air	360
Lower flammability limit %v/v i.air	13.5
Stability	volatile
Solubility	complete in water, alcohols, ketones, ether
Toxicity data	
LD oral	5700 mg/kg man
LD ₅₀ oral	5400 mg/kg rat
LD ₅₀ subcutaneous	3230 mg/kg mouse
Inhalation LD for rat	4000 ppm (4 h)
Irritant to skin and mucosa	
Exposure limits UK	500 (200) mg/m ³ (ppm)
Exposure limits USA	492 (200) mg/m ³ (ppm)
LC ₅₀ for fish	4560 mg/litre

Antimicrobial effectiveness/applications

Compared with ethanol, 1-propanol is effective at lower but still relatively high concentrations, e.g. 50–60% for hand disinfection. The presence of water is essential for the efficacy.

1-Propanol is a suitable active ingredient in disinfectants, e.g. for the hands; it may also be used as a preservative for cosmetic products.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.3. 2-Propanol
Chemical formula	C ₃ H ₈ O
Structural formula	(CH ₃) ₂ CH-OH
Molecular mass	60.10
CAS-No.	67-63-0
EC-No.	200-661-7
Synonym/common name	isopropanol, isopropyl alcohol, dimethyl carbinol
Supplier	BP, EXXON, HOUGHTON, HULS, SHELL, UNION CARBIDE

Chemical and physical properties

Appearance	clear, colourless, burnable fluid with a sharp aromatic odour
Content %	99
Boiling point/range °C (101 kPa)	82–83
Melting point °C	–88
Density g/ml (20°C)	0.785–0.786
Vapour pressure hPa (20°C)	41
Viscosity mPas (20°C)	2.2–2.4
Surface tension mN/m (20°C)	22.7
Refractive index nD (20°C)	1.377
Flash point °C	12
Auto ignition temperature °C	399–425
Upper flammability limit %v/v i.air	12
Lower flammability limit %v/v i.air	2.0–2.5
Stability	volatile
Solubility	complete in water, alcohols, ketones, ether
Toxicity data	
LD oral	8600 mg/kg man
LD ₅₀ oral	5850 mg/kg rat
Irritant to skin and mucosa	
Neither carcinogenic nor hepatotoxic.	

Metabolism: after incorporation (inhalatively or orally) 64–84% are transferred to acetone by oxidation.	
Exposure limits German/UK	980 (400) mg/m ³ (ppm)
USA	983 (400) mg/m ³ (ppm)
100 ppm are registered by sensitive sense of smell	
LC ₅₀ for fish	8970 mg/litre

Antimicrobial effectiveness/applications

2-Propanol and 1-propanol (1.2.) are the highest alcohols which are miscible with water. The presence of water is essential for the effectiveness of 2-propanol, too. Most effective are concentrations of approx. 50%. Investigations of Powell (1945) have shown that *Stahylococcus aureus* was killed within 1 minute at 20°C in 50 to 91% 2-propanol solutions. *Escherichia coli* was killed by a 5-minute exposure at 20°C to $\geq 30\%$ solutions. The data reported by Wallhäusser (1984) for ethanol (1.1.) demonstrate the higher efficacy of 2-propanol which in the meantime has become a proved alternative to ethanol for use in disinfectants, e.g. in hand rinses, and as a preservative in cosmetics.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.4. Benzyl alcohol
Chemical formula	C ₇ H ₈ O
Structural formula	C ₆ H ₅ -CH ₂ -OH
Molecular mass	108.13
CAS-No.	100-51-6
EC-No.	202-859-9; EEC-no. 34
Synonym/common name	phenyl methanol, phenyl carbinol
Supplier	BAYER, Elf ATOCHEM, HAARMANN & REIMER
Chemical and physical properties	
Appearance	clear, colourless, burnable fluid with a slight alcoholic odour
Boiling point/range °C (101 kPa)	205–206
Melting point °C	–15.7
Density g/ml (20°C)	1.050
Vapour pressure hPa (20°C)	0.13
Viscosity mPas (20°C)	6.0
Surface tension mN/m (20°C)	38.8
Refractive index nD (20°C)	1.540–1.541
Flash point °C	94
Auto ignition temperature °C	436
Upper flammability limit %v/v i.air	13
Lower flammability limit %v/v i.air	1.3
Stability	slow oxidation in contact with oxgen to benzaldehyde
Solubility g/l (20°C)	in water 38, highly soluble in alcohols, ketones, ether, aromatic solvents, oils
Toxicity data	
LD oral	500 mg/kg man
LD ₅₀ oral	1230 mg/kg rat
	1580 mg/kg mouse
	1940 mg/kg rabbit
LD ₅₀ intraperitoneal	800 mg/kg guinea pig
LD ₅₀ intravenous	300 mg/kg rat
LD ₅₀ percutaneous	> 5 ml/kg guinea pig
	see also Wollmann et al., 1963

Benzyl alcohol is irritant to skin and mucosa, but not a skin sensitiser and not mutagenic. Metabolism: oxidation to benzoic acid and excretion as hippuric acid.

Antimicrobial effectiveness/applications

Benzyl alcohol exhibits a broad spectrum of effectiveness which covers bacteria, yeasts and moulds. However, the activity against moulds is more intense than against bacteria (see Table 3.). The bactericidal action is slow.

Benzyl alcohol is listed in the EC positive list of preservatives for cosmetic products (maximum concentration for application: 10.000 mg/litre); as a preservative it may be useful also in pharmaceutical preparations. Percentage of use in US cosmetic formulations: 0.32%. The activity of benzyl alcohol is not very much affected by the pH and the composition of the medium to be protected. As an auxiliary solvent with antimicrobial efficacy benzyl alcohol is used in preservative compositions for industrial fluids (Paulus et al., 1970a). A well-known preservative for cosmetics and industrial fluids is benzyl alcohol mono(poly)hemiformal (Paulus, 1976) which is a formaldehyde releasing compound and therefore listed under 3.1.2.

Table 3 Minimum inhibition concentrations (MIC) of benzyl alcohol in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	5000
<i>Escherichia coli</i>	4000
<i>Pseudomonas aeruginosa</i>	3000
<i>Pseudomonas fluorescens</i>	6000
<i>Staphylococcus aureus</i>	5000
Formaldehyde resistant bacteria	5000
<i>Candida albicans</i>	3500
<i>Candida crusei</i>	6000
<i>Aureobasidium pullulans</i>	1500
<i>Chaetomium globosum</i>	2500
<i>Trichoderma viride</i>	4500
<i>Trichophyton mentagrophytes</i>	3000

Microbicide group (substance class)

Chemical name

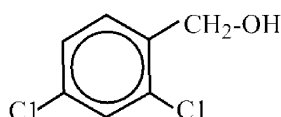
Chemical formula

Structural formula

1. ALCOHOLS

1.5. 2,4-Dichlorobenzyl alcohol

$C_7H_6Cl_2O$



Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

177.03

1777-82-8

217-210-5; EEC-no.22

2,4-dichlorophenyl methanol

BASF, BOOTS MICROCHECK, INOLEX CHEMICAL

Chemical and physical properties

Appearance

Content %

Boiling point/range °C (101 kPa)

Melting point °C

Flash point °C

Stability

Solubility g/l (20°C)

white to yellow crystals

99

150

55–58

> 110

oxidisable to 2,4-dichloro benzaldehyde

approx. 1 in water, highly soluble in organic solvents

Toxicity data

LD₅₀ oral

LD₅₀ subcutaneous

2300 mg/kg mouse

1700 mg/kg mouse

After dermal adsorption approx. 90% are excreted with the urine within 96 h. 2,4-Dichlorobenzyl alcohol does not cause skin irritation and sensitization, is not mutagenic and not teratogenic (Wallhäüßer, 1984), however, is irritant to mucous membranes.

Ecotoxicity (source BASF Specialty Chemicals)

LC ₅₀ for fish (96 h)	10–100 mg/l
LC ₅₀ for Daphniae (48 h)	10–100 mg/l

Antimicrobial effectiveness/applications

2,4-dichlorobenzyl alcohol is much more effective than benzyl alcohol (1.4.) and additionally exhibits a more equalized spectrum of effectiveness (see Table 4). Optimum pH range: 4–10.

The solubility of 2,4-dichlorobenzyl alcohol in water is poor; in aqueous systems it tends to migrate into the organic phase. However, it is compatible with most used cosmetic and pharmaceutical ingredients and listed in the EC positive list of preservatives for cosmetic products (maximum concentration for the application: 0.15%). Main application: in hand disinfectants. – Percentage of use in US cosmetic formulations: 0.06%.

Table 4 Minimum inhibition concentrations (MIC) of 2,4-Dichloro benzyl alcohol in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	500
<i>Pseudomonas aeruginosa</i>	1500
<i>Staphylococcus aureus</i>	500
<i>Aspergillus niger</i>	500
<i>Chaetomium globosum</i>	200
<i>Penicillium glaucum</i>	500
<i>Candida albicans</i>	500

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.6. 2-Phenyl-ethan-1-ol
Chemical formula	C ₈ H ₁₀ O
Structural formula	C ₆ H ₅ -(CH ₂) ₂ -OH
Molecular mass	122.17
CAS-No.	60-12-8
EC-No.	200-456-7
Synonym/common name	phenylethyl alcohol, benzylcarbinol, phenethyl alcohol
Supplier	BASF
Chemical and physical properties	
Appearance	clear, colourless fluid smelling of the attar of roses
Content %	99
Boiling point/range °C (101 kPa)	215–217
Melting point °C	–27
Density g/ml (20°C)	1.020
Refractive index nD (20°C)	1.532
Flash point °C	102
Stability	sensitive to oxidizing agents
Solubility g/l (20°C)	approx. 20 in water; complete in alcohols, ketones, ether; soluble in fatty oils; poor solubility in mineral oils
Toxicity data	
LD ₅₀ oral	1790 mg/kg rat
LD ₅₀ cutaneous	5–10 ml/kg guinea pig
No-effect level for rats (90-day-feeding-test): 0.25–0.50 ml/kg body weight/day.	

Antimicrobial effectiveness/applications

As the effectiveness against bacteria is weak, weaker than the activity against moulds (see Table 5), 2-phenyl-ethan-1-ol is in general applied in combination with other microbicides, e.g. with p-hydroxy-benzoic acid esters (8.1.11.), quaternary ammonium compounds (18.1.), p-chloro-m-cresol (7.3.1.) for the preservation of cosmetic and pharmaceutical products. Remarkable synergisms are observed. Examinations of Richards & McBride (1973) prove that 2-phenyl-ethan-1-ol causes changes in the permeability of the cell membrane of *Pseudomonas aeruginosa* thus facilitating the permeation of other antimicrobially active substances. – Especially effective is 2-phenyl-ethan-1-ol in acidic media.

Table 5 Minimum inhibition concentrations (MIC) of 2-Phenyl ethanol in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	2500
<i>Pseudomonas aeruginosa</i>	3500
<i>Staphylococcus aureus</i>	4750
<i>Aspergillus niger</i>	2750
<i>Chaetomium globosum</i>	2000
<i>Penicillium glaucum</i>	2750
<i>Candida albicans</i>	2500

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.7. 2-Phenoxy-ethan-1-ol
Chemical formula	$C_8H_{10}O_2$
Structural formula	$C_6H_5-O-(CH_2)_2-OH$
Molecular mass	138.17
CAS-No.	122-99-6
EC-No.	204-589-7; EEC-no. 29
Registrations: world-wide approvals for use in personal care products	
Synonym/common name	ethylen glycol monophenyl ether, phenoxyethyl alcohol, Phenyl Cellosolve, Dowanol EPh, Phenoxetol, Protectol PE
Supplier	BASF, CLARIANT-NIPA, DOW, HAARMANN & REIMER
Chemical and physical properties	
Appearance	clear, colourless, slightly viscous fluid with a pleasant odour
Content %	100
Boiling point/range °C (101 kPa)	245.2
Melting point °C	14
Density g/ml (20°C)	1.1094
Vapour pressure hPa (20°C)	< 0.01
Viscosity mPa·s (20°C)	approx. 24
Refractive index nD (20°C)	1.538
Flash point °C	107
Stability	oxidation leads to the corresponding aldehyde and carboxylic acid
Solubility g/l (20°C)	26 in water, 7 in mineral oil, 39 in isopropyl palmitate; miscible with alcohol, glycerine, propylene glycol, ether, benzene
Partition coefficients	2.9 in isopropyl palmitate-water 2.6 in peanut oil-water 0.3 in mineral oil-water
Toxicity data	
LD ₅₀ oral	1.26–2.33 ml/kg rat
LD ₅₀ dermal	> 2000 mg/kg rabbit

Does not cause skin irritation. No sensitisation was observed in corresponding tests with guinea pigs. – Undiluted 2-phenoxy-ethanol is an eye irritant; solutions in water do not cause irritation.

Ecotoxicity (source BASF Specialty Chemicals)

EC ₁₀ for bacteria (17h)	> 100 mg/l
LD ₅₀ for <i>Leuciscus idus</i>	> 100 mg/l
EC ₅₀ (acute) for <i>Daphnia</i> (48 h)	> 100 mg/l

Antimicrobial effectiveness/applications

Although there are reports about the antimicrobial properties of 2-phenoxy-ethanol since 1944, e.g. Berry, the compound is nowadays of special interest as a preservative, because of its chemical and physical properties, preferably in combination with other preservatives, such as p-hydroxy-benzoic acid esters (8.1.11.), 1,2-dibromo-2,4-dicyanobutane (17.18.) and others. Such combinations may substitute highly effective but controversial microbicides especially in cosmetic and pharmaceutical products. In this connection the fact that 2-phenoxy-ethanol additionally is a good solvent plays an important role, as well as the partition coefficients which demonstrate that the distribution of 2-phenoxy-ethanol between organic phases and aqueous phases will be relatively even. The stability of 2-phenoxy-ethanol guarantees that a concentration of the microbicide which proved to be sufficiently effective as a preservative, will not decrease with time, and therefore will be able to control recontaminations during storage or use. The minimum inhibition concentrations listed in Table 6. demonstrate that 2-phenoxy-ethanol is preferably active as a fungicide. Flores et al. (1997) found Bacilli, e.g. *B. brevis*, *B. lentus*, *B. megaterium*, Micrococci, e.g. *M. roseus*, *M. sedentarius*, and Staphylococci, e.g. *S. aureus*, *S. epidermidis*, *S. saprophyticus*, to be resistant to 0.4% 2-phenoxy-ethanol. The results underline that the use of mixtures of preservatives is essential, in order to obtain good protection of aqueous functional fluids such as cosmetics, or metal working fluids. In the EC list of preservatives for cosmetic products 2-phenoxy-ethanol is mentioned with a maximum authorized concentration of 1% (10000 mg/litre). – Percentage of use in US cosmetic formulations: 1.87%. The addition of formaline to 2-phenoxy-ethanol leads to 2-phenoxy-ethanol hemiformal (3.1.3.) which has to be regarded as a formaldehyde releasing compound, and consequently disposes of a higher antimicrobial efficacy than 2-phenoxy-ethanol alone.

Table 6 Minimum inhibition concentrations (MIC) of 2-phenoxy-ethanol. Source: BASF Specialty Chemicals

Organism		M.I.C. (%)
Gram positive bacteria		
<i>Bacillus subtilis</i>	NCTC 10073	1.00
<i>Staphylococcus aureus</i>	ATCC 6538	0.75
<i>Staphylococcus epidermidis</i>	NCIB 9518	0.64
<i>Streptococcus faecalis</i>	NCTC 8213	0.32
Gram negative bacteria		
<i>Enterobacter cloacae</i>	(Pre. ref. 146)	0.32
<i>Escherichia coli</i>	NCIB 9517	0.32
<i>Klebsiella aerogenes</i>	NCTC 418	0.50
<i>Proteus vulgaris</i>	ATCC 14153	0.75
<i>Pseudomonas aeruginosa</i>	NCTC 6750	1.00
<i>Burkholderia cepacia</i>	NCIB 9085	1.00
<i>Pseudomonas fluorescens</i>	NCIB 9046	1.50
<i>Pseudomonas putida</i>	NCIB 9034	0.32
<i>Pseudomonas stutzeri</i>	NCIB 9040	0.32
<i>Salmonella typhimurium</i>	NCTC 74	0.32
<i>Serratia marcescens</i>	(Industrial isolate)	0.32
Yeasts		
<i>Saccharomyces cerevisiae</i>	NCYC 87	0.25
<i>Candida albicans</i>	ATCC 10231	0.32
<i>Candida tropicalis</i>	(Industrial isolate)	0.32
<i>Spoilage Yeast</i>	Y67	0.32
Moulds		
<i>Aspergillus niger</i>	ATCC 16404	0.25
<i>Chaetomium globosum</i>	IMI 45550	0.16
<i>Cladosporium herbarum</i>	(Industrial isolate)	0.16
<i>Penicillium funiculosum</i>	IMI 87160	0.06
<i>Stachybotrys atra</i>	IMI 82021	0.06
<i>Trichoderma viride</i>	(Industrial isolate)	0.25

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.8. 3-Phenyl-propan-1-ol
Chemical formula	$C_9H_{12}O$
Structural formula	$C_6H_5-(CH_2)_3-OH$
Molecular mass	136.20
CAS-No.	122-97-4
EC-No.	204-587-6
Synonym/common name	3-phenyl-propyl alcohol, hydrocinnamyl alcohol, phenethylcarbinol
Supplier	BASF, GIVAUDAN, HAARMANN & REIMER
Chemical and physical properties	
Appearance	clear, colourless fluid of sweet odour and taste
Content %	> 98
Boiling point/range °C (1,6 kPa)	119–121
Density g/ml (20°C)	1.001
Vapour pressure hPa (20°C)	
Refractive index nD (20°C)	1.527
Solubility	poorly soluble in water, highly soluble in polar and non polar solvents

Toxicity data

According to FDA (121.1164) the compound is generally recognized as safe (GRAS).

Antimicrobial effectiveness/applications

3-Phenyl-propan-1-ol disposes of a broad spectrum of antimicrobial effectiveness; it is recommended for the protection of cosmetic products; addition rate 0.4% (Fiedler, 1989).

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.9. 1-Phenoxy-propan-2-ol
Chemical formula	$C_9H_{12}O_2$
Structural formula	$C_6H_5-O-CH_2-CHOH-CH_3$
Molecular mass	152.2
CAS-No.	770-35-4
EC-No.	212-222-7; EEC-no. 43
Synonym/common name	Propylene glycol monophenylether, 2-(hydroxypropyl) phenylether, phenoxy-isopropanol, Dowanol PPh, Propylenephenoxetol
Supplier	BASF, CLARIANT-NIPA, DOW
Chemical and physical properties	
Appearance	clear, colourless fluid
Content %	≥99
Boiling point/range °C (101 kPa)	242
Solidification point °C	13
Density g/ml (20°C)	1.062
Viscosity mPa·s (20°C)	35.39
Refractive index nD (20°C)	1.52397
Flash point °C	127
Auto ignition temperature °C	135
Upper flammability limit %v/v i.air	9.4
Lower flammability limit %v/v i.air	0.7
Stability	chemically inert, non-volatile, fully stable over a wide pH and temperature range
Solubility g/l (20°C)	14 in water; soluble in polar and non-polar solvents; insoluble in mineral oil

Toxicity data

According to the indications of Fiedler (1989) the special merits of phenoxyisopropanol are good skin and eye compatibility.

Antimicrobial effectiveness/applications

Because of its broad spectrum of effectiveness and its favourable dermatological properties 1-phenoxy-propan-2-ol is mentioned in the EC list of preservatives allowed for the protection of cosmetic products with a maximum concentration of 1% (for rinse-off products only).

Percentage of use in US formulations: 0.02%.

A liquid 99% mixture of

1-phenoxy-propan-2-ol (1.8.) plus

2-phenoxy-propan-1-ol (CAS-No. 4169-04-4; EC-No. 224-027-4)

serves as a co-preservative for functional fluids, too. – Log POW: 1.5.

Common name: Phenoxypropanol

Supplier: BASF, CLARIANT-NIPA

As can be seen from the minimum inhibition concentrations listed in Table 7, the mixture exhibits a moderate antimicrobial activity; in consequence it is mainly used in combination with other microbicides. Its activity is largely independent of pH.

Toxicity data for the Phenoxypropanol mixture (Source: BASF Specialty Chemicals)

LD ₅₀ oral	> 2000 mg/kg rat
LD ₅₀ dermal	> 2000 mg/kg rabbit
Ecotoxicity	
EC ₁₀ for bacteria (17 h)	> 100 mg/l
LC ₅₀ for <i>Leuciscus idus</i> (96 h)	> 100 mg/l
EC ₅₀ (acute) for <i>Daphniae</i> (48 h)	> 100 mg/l

Table 7 Minimum inhibition concentrations (MIC) of phenoxypropanol (Source: BASF specialty chemicals)

Test organism	MIC (%)
<i>Staphylococcus aureus</i>	ATCC 6538 0.50
<i>Escherichia coli</i>	ATCC 11229 0.50
<i>Proteus mirabilis</i>	ATCC 14153 0.50
<i>Pseudomonas aeruginosa</i>	ATCC 15442 1.00
<i>Candida albicans</i>	ATCC 10231 0.75

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.10. 1,1,1-Trichloro-2-methyl-2-propanol
Chemical formula	C ₄ H ₇ Cl ₃ O
Structural formula	CCl ₃ -C(CH ₃) ₂ -OH
Molecular mass	177,46
CAS-No.	6001-64-5
EC-No.	200-317-6; EEC-no. 11
Synonym/common name	trichlorisobutyl alcohol, trichloro-tert.butanol, Chlorobutanol, Chlorbutol
Supplier	ALDRICH CHEMICALS, MERCK INC.
Chemical and physical properties	
Appearance	colourless, sublimating crystals with an odour similar to camphor
Content %	98 (hydrate)
Boiling point/range °C (101 kPa)	167
Melting point °C	77–79

Flash point °C	> 100
Stability	decomposition by alkalies and heat, stable up to pH 4
Solubility g/l (20°C)	8 i. water; highly soluble in alcohols, ketones, ether and oils
Toxicity data	
Irritant to skin and mucous membranes	
Primary irritation	rabbit skin 850 µg – mild rabbit eye 9180 µg/30 sec – mild
Incorporation orally, dermally, or by inhalation may affect the central nervous system.	

Antimicrobial effectiveness/applications

The activity spectrum of 1,1,1-trichloro-methyl-2-propanol comprises bacteria and fungi. It may be used as a preservative for cosmetic and pharmaceutical products. In the EC list of preservatives which cosmetic products may contain it is mentioned under the name 'Chlorobutanolum'. The authorized concentration is 0,5%; excluded is the application in sprays. Printed label warning: Contains chlorobutanol. – Percentage of use in US cosmetic formulations: 0.005%.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.11. 3-Iodo-2-propin-1-ol (IPA)
Chemical formula	C ₃ H ₃ IO
Structural formula	I-C≡C-CH ₂ -OH
Molecular mass	165.97
CAS-No.	1725-82-2
EC-No.	unknown
Synonym/common name	3-iodopropargyl alcohol, IPA
Chemical and physical properties	
Appearance	yellowish solid of an unpleasant odour
Melting point °C	41–42
Stability	not stable in hot water: partial transformation to 1,1,2-triiodo-propene(1)-3-ol (1.12.) which exhibits strong antimicrobial activity, too
Solubility g/l (20°C)	1.5 in water; highly soluble in polar and aromatic solvents

Table 8 Minimum inhibition concentrations (MIC) of IPA in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	5
<i>Aspergillus niger</i>	5
<i>Aureobasidium pullulans</i>	5
<i>Chaetomium globosum</i>	5
<i>Cladosporium cladosporioides</i>	2
<i>Coniophora puteana</i>	1
<i>Lentinus tigrinus</i>	5
<i>Penicillium glaucum</i>	5
<i>Polyporus versicolor</i>	2
<i>Sclerophoma pityophila</i>	2
<i>Trichoderma viride</i>	10
<i>Candida albicans</i>	20
<i>Candida krusei</i>	20
<i>Rhodotorula mucilaginosa</i>	10
<i>Rhodotorula glutinis</i>	15
<i>Sporobolomyces roseus</i>	15
<i>Torula rubra</i>	10
<i>Escherichia coli</i>	100
Formaldehyde resistant bacteria	15
<i>Staphylococcus aureus</i>	150

Toxicity data

LD₅₀ oral 140–170 mg/kg rat

Irritant and corrosive to skin and mucosa.

Non-mutagenic (Salmonella microsome test).

Antimicrobial effectiveness/applications

IPA exhibits an extremely broad activity spectrum covering fungi, yeasts and bacteria. However, IPA is approximately 10 times more effective against fungi and yeasts than against bacteria (see Table 8).

3-Iodopropargyl derivatives (11.1.–11.3 and 3.1.6.) which may be regarded as IPA extricating compounds, e.g. 3-iodopropargyl-N-butylcarbamate, are used mainly in fungicidal finishing of materials; but the less complex compound, IPA, although very effective, is unsuitable for that, being volatile and easily leached. IPA has also acquired no appreciable importance as a preservative. Its high price in comparison with other preservatives, an irritating effect on the skin, troublesome odour and a toxicity profile which is, on the whole, unfavourable, have prevented this.

Microbicide group (substance class)

1. ALCOHOLS

Chemical name

1.12. 1,1,2-Triiodo-propene(1)-3-ol

Chemical formula

C₃H₃I₃O

Structural formula

I₂C = CI-CH₂-OH

Molecular mass

435.77

CAS-No.

42778-72-3

EC-No.

unknown

Synonym/common name

2,3,3-triiodallyl alcohol, TIAA

Chemical and physical properties

Appearance

pale, yellowish to greyish brown crystalline powder

Melting point °C

150–152

Solubility

highly soluble in dimethyl sulphoxide, dimethyl formamide, dioxane, cyclohexane; moderately soluble in methanol, ethanol, acetone, ethyl acetate, isopropanol; slightly soluble in ethylene glycol, isobutanol; hardly soluble in water

Toxicity data

LD₅₀ oral > 5000 mg/kg mouse

Table 9 Minimum inhibition concentrations (MIC) of tri-iodallyl alcohol in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	1
<i>Aspergillus niger</i>	3.5
<i>Aureobasidium pullulans</i>	1
<i>Chaetomium globosum</i>	7.5
<i>Coniophora puteana</i>	0.5
<i>Lentinus tigrinus</i>	1
<i>Penicillium glaucum</i>	2
<i>Polyporus versicolor</i>	2
<i>Sclerophoma pityophila</i>	2
<i>Trichoderma viride</i>	10
<i>Escherichia coli</i>	50
<i>Staphylococcus aureus</i>	10
<i>Slime bacteria</i> ^a	2.5

^a Described by Kato & Fukumura (1962)

Antimicrobial effectiveness/applications

TIAA performs as a broad spectrum microbicide which may be used in anti-mould and anti-sapstain agents and in wood preservatives because of its extraordinary efficacy against wood destroying fungi (Lee et al., 1990).

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.13. 1,2-Ethanediol
Chemical formula	$C_2H_6O_2$
Structural formula	HO-CH ₂ -CH ₂ -OH
Molecular mass	62.07
CAS-No.	107-21-1
EC-No.	203-473-3
Synonym/common name	ethylene glycol, dioxyethane
Supplier	BASF, ENICHEM, HOUGHTON, SHELL, UNION CARBIDE

Chemical and physical properties

Appearance	colourless, odourless fluid with a sweet taste
Content (%)	> 98
Boiling point/range °C (101 kPa)	193–197
Melting point °C	–11.5 to –12.7
Density g/ml (20°C)	1.115
Vapour pressure hPa (20°C)	0.07
Viscosity mPas (20°C)	13.7–19.9
Surface tension mN/m (20°C)	48.49
Refractive index nD (20°C)	1.4318
Flash point °C	111
Auto ignition temperature °C	410
Upper flammability limit %v/v i.air	15.3
Lower flammability limit %v/v i.air	3.2
Solubility	complete in water and lower aliphatic alcohols and ketones; insoluble in non-polar solvents; due to its well balanced molecule structure (two hydrophobic CH ₂ -groups and two hydrophilic OH-groups) 1,2-ethanediol is able to dissolve inorganic as well as organic substances
Log POW	–1.93

Toxicity data

LD ₅₀ oral	8.540 mg/kg rat 6.610 mg/kg guinea pig 2.700 mg/kg mouse
LD subcutaneous	
Inhalation of 1,2-ethanediol causes respiratory tract irritation.	
Exposure limits (occupational)	German 26 (10 mg/m ³ (ppm))

Ecotoxicity: 1,2-Ethanediol is known to be relatively non-persistent (aerobic biodegradation half-lives ranging from 2 to 18 days), and to dispose of low aquatic toxicity.

LC₅₀ for rainbow trout (exposure 96 h): 17.800 mg/l.

The suggestion that 1,2-ethanediol because of its low log octanol/water partition coefficient does not accumulate to significant levels in the tissues of aquatic biota, is corroborated by long term studies with crayfish (Kent et al., 1999).

Antimicrobial effectiveness/application

1,2-Ethanediol does not exhibit substantial antimicrobial activity. However, due to its hygroscopic (water binding) properties it may affect microbial proliferation, e.g. in cosmetics and toiletries, by reducing the available water in the corresponding formulations. Also remarkable is the fact that the addition of formaldehyde (2.1.) to 1,2-ethanediol leads to the mono- and bis-hemiformal of 1,2-ethanediol (3.1.4.), compounds which easily release considerable amounts (32.6/42.2%) of formaldehyde.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.14. 1,2-Propanediol
Chemical formula	$C_3H_8O_2$
Structural formula	$CH_3-CH(OH)-CH_2-OH$
Molecular mass	76.10
CAS-No.	57-55-6
EC-No.	200-338-0
Synonym/common name	propylene glycol
Supplier	ARCH, BASF, HOUGHTON, HULS
Chemical and physical properties	
Appearance	colourless, nearly odourless oily fluid
Content (%)	~99
Boiling point/range °C (101 kPa)	186–190
Solidification point °C	–59
Density g/ml (20°C)	1.036
Vapour pressure hPa (20°C)	0.27
Viscosity mPas (20°C)	55–60
Surface tension mN/m (20°C)	38
Refractive index	1.433
Flash point °C	103
Auto ignition temperature °C	410
Upper flammability limit %v/v i.air	12.6
Lower flammability limit %v/v i.air	2.6
Solubility	complete in water; highly soluble in many organic solvents
Log POW	–0.3
Toxicity data	
According to the following data 1,2-propanediol is mostly regarded as non-toxic.	
LD ₅₀ oral	28.280 mg/kg rat
subcutaneous	22.000 mg/kg rat
LD ₅₀ intravenous	7.000 mg/kg rat
LD ₅₀ dermal (exposure 24 h)	> 20 ml/kg rabbit
Rabbit skin (exposure 4 h): no skin irritation, but slight eye irritation.	
Ecotoxicity: Similar to 1,2-ethanediol (1.13.). Relatively non-persistent and of low aquatic toxicity (Kent et al., 1999).	

Antimicrobial effectiveness/application

1,2-Propanediol as well as 1,3-butanediol (1.15.) achieve antimicrobial activity at concentrations > 10%; additionally both solvents increase the bioavailability of microbicides of low water solubility, e.g. p-hydroxybenzoates (8.1.11.), in the water phase.

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.15. 1,3-Butanediol
Chemical formula	$C_4H_{10}O_2$
Structural formula	$CH_3-CH_2(OH)-CH_2-CH_2-OH$
Molecular mass	90.12
CAS-No.	107-88-0
EC-No.	203-529-7
Synonym/common name	1,3-butyleneglycol
Supplier	HÜLS, SIGMA ALDRICH
Chemical and physical properties	
Appearance	clear, colourless and odourless fluid with a sweet taste
Content (%)	~99

Boiling point/range °C (101 kPa)	204–207
Solidification point °C	> –50
Density g/ml (20°C)	1.004
Vapour pressure hPa (20°C)	0.02
Viscosity mPas (20°C)	137
Surface tension mN/m (20°C)	37.8
Refractive index nD (20°C)	1.440
Flash point °C	109
Auto ignition temperature °C	440
Upper flammability limit %v/v i.air	9.4
Lower flammability limit %v/v i.air	1.8
Solubility	highly soluble in water and alcohols

Toxicity data

LD ₅₀ oral	29.4 ml/kg rat

1,3-butanediol is neither irritant to the skin nor to mucous membranes, and is regarded as a non-toxic substance.

Antimicrobial effectiveness/application

See 1,2-propanediol (1.14.).

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.16. 2-(Butoxyethoxy)ethanol
Chemical formula	C ₈ H ₁₈ O ₃
Structural formula	HO-(CH ₂) ₂ -O-(CH ₂) ₂ -O-C ₄ H ₉
Molecular mass	162.23
CAS-No.	112-34-5
EC-No.	203-961-6
Synonym/common name	butyldiglycol, Butylcarbitol, Dowanol DB
Supplier	DOW

Chemical and physical properties

Appearance	clear, odourless fluid
Content (%)	approx. 100
Boiling point/range °C (101 kPa)	230.4
Solidification point °C	–68.1
Density g/ml (20°C)	0.9536
Viscosity mPas (20°C)	4.2
Refractive index nD (27°C)	1.4258
Flash point °C	98
Solubility	complete in water, highly soluble in oils and other organic solvents

Toxicity data

LD ₅₀ oral	6.560 mg/kg rat
	2.000 mg/kg guinea pig
LD ₅₀ dermal (24 h)	4.12 ml/kg rabbit
LD ₅₀ intraperitoneal	850 mg/kg mouse

Non-irritant to the skin, but to the eyes of rabbits. – Non-teratogenic.

Antimicrobial effectiveness/application

Butylcarbitol is used as a solvent or a solution mediator, e.g. for fats and oils, in the formulation of cosmetic products. Its antimicrobial efficacy is not noteworthy. Nevertheless it is mentioned here, as it is easily transformed to a solvent with antimicrobial activity by the addition of 1M paraformaldehyde (2.1a.) which leads to the corresponding hemiformal (3.1.5.).

Microbicide group (substance class)	1. ALCOHOLS
Chemical name	1.17. 3-(4-Chlorophenoxy)-1,2-propanediol
Chemical formula	$C_9H_{11}ClO_3$
Structural formula	
Molecular mass	202.64
CAS-No.	104-29-0
EEC-No.	50
Synonym/common name	p-chlorophenyl- α -glycerolether, Chlorphenesin
Supplier	LENTIA
Chemical and physical properties	
Appearance	white crystals with faint phenolic odour
Content (%)	approx.100
Boiling point/range °C (2.6 kPa)	214–215
Melting point °C	80
Stability	stable under normal conditions; non-volatile in steam; unaffected by dilute acids and alkalies; light-stable; thermostable below 100°C
Solubility g/l (25°C)	6 in H ₂ O; soluble in organic solvents, preferably in alcohols
Toxicity data (source: Kabara)	
LD ₅₀ subcutaneous	930 mg/kg mouse
Mildly irritant to skin and eyes.	

Antimicrobial effectiveness/applications

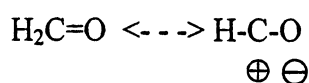
The α -glycerolether is prepared by condensing equimolar amounts of 4-chlorophenol (7.5.1.) and 2,3-epoxy-1-propanol (Glycidol) in the presence of a tertiary amine or a QAC as catalyst. Minimum inhibition concentrations (source: Kabara, 1984, e.g. 1250 mg/l for *Staphylococcus aureus*, 2500 mg/l for *Pseudomonas aeruginosa* and *Aspergillus niger*, 1250 mg for *Candida albicans*) present Chlorphenesin as a microbicide of moderate efficacy. Optimum pH range: 4–6. Chlorphenesin is used as a preservative in cosmetic and pharmaceutical products. In the EEC Cosmetic Directive it is listed with a maximum permitted concentration of 0.3%.

2 Aldehydes

As is specified in Part One, Chapter 2, Figures 6 and 7, aldehydes belong to the group of electrophilic active agents, which due to the electron deficiency at the carbonyl carbon atom (Figure 1) can react with nucleophilic cell entities and thus exert antimicrobial activity. Dialdehydes dispose of two electrophilic centers able to interact with microbial nucleophilic cell constituents and to undergo manifold connection reactions.

Examples of nucleophilic reaction partners in the cell are amino and thiol groups, as well as the amide groups of amino acids of proteins (Figure 2). These in turn are components of enzymes, which are inactivated by the reaction of their nucleophilic groups with aldehydes.

Formaldehyde (2.1.) is the most reactive and thus most effective among monoaldehydes. Glutaric aldehyde (2.5.) is the most effective dialdehyde. Other dialdehydes such as glyoxal (2.3.), malonic aldehyde, succinic aldehyde (2.4.) and adipic aldehyde have not attained appreciable significance as microbicides although possessing sporicidal effects. Higher aliphatic aldehydes with molecular weights higher than that of the adipic aldehyde display no noticeable microbicidal effect. Aromatic mono- and dialdehydes, too, exhibit microbicidal effective-



$\oplus$ = electron deficiency
 $\ominus$ = electron surplus

Figure 1 Electron hole at the carbonyl carbon atom of aldehydes.

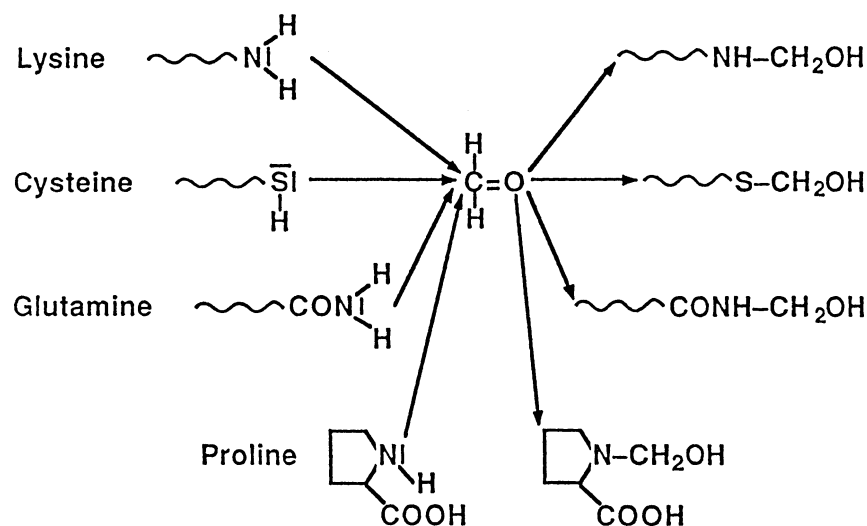


Figure 2 Reaction of aldehydes with amino acids.

ness. Up to now these aldehydes have not gained importance in practice, although several authors emphasize their utility as preservatives and active ingredients in sterilizing and disinfecting compositions (Bruckner et al., 1998; Maddox, 1988; Sharma, 1989).

Because of the mechanism mentioned above the aldehydes with microbicidal effect are distinguished by broad spectra of effectiveness which also cover viri and spores. Since only few sporicides are found among microbicides, the sporicidal effect of aldehydes, particularly that of glutaric aldehyde is of special interest. Aldehydes are above all used for disinfecting purposes; but also for preservation, then chiefly in the form of so-called aldehyde releasing compounds (3., 4., 5., 6.)

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

Synonym/common name

Chemical and physical properties

Appearance

Boiling point/range °C (101 kPa)

Solidification point °C-118

Flash point °C

Stability

Solubility

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

2. ALDEHYDES

2.1. Formaldehyde

CH₂O

O = CH₂

30.03

50-00-0

methanal, methylene oxide

reactive, colourless, inflammable gas of pungent odour; the gas obeys the ideal gas laws

-19

-118

~300

the dry gas tends to polymerize slowly; as a reductive agent formaldehyde is easily oxidized, e.g by hydrogen peroxide, iodine, sodium permanganate; it reacts with ammonia and proteins under inactivation; the influence of alkali leads to disproportionate of formaldehyde to methanol and formic acid (6.1.1.)

soluble in H₂O (up to 55%), forming formalin (2.1c.), soluble in alcohols, forming hemiformals (3.1.), soluble in polar solvents

2.1 a. Paraformaldehyde

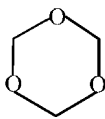
(CH₂O)_n n = 8-100

H(CHOH)_nOH

30.03

30525-89-4

200-001-8; EEC-no. 5

EPA/FIFRA	approval for antimicrobial applications – subject to registration or re-registration
Synonym/common name	poly-methanal, polyoxmethylene, Formagene, Paraform
Supplier	BASF, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	solid inflammable substance; paraformaldehyde is a mixture of the polymerisation products of formaldehyde, containing 91–99% formaldehyde, and small amounts of H ₂ O which are added to chains of formaldehyde, thus disrupting the polymerization; common grades include flaked, powdered and granular white crystalline materials with the distinct odour of formaldehyde
Content %	ca. 95
Melting point °C	163–165
Density g/ml (20°C)	0.88
Flash point °C	70
Stability	depolymerizes in acids, alkalis, and hot water; depolymerisation of paraformaldehyde also takes place in polar solvents, very rapidly if heat is involved and traces of an alkaline material, e.g. potassium carbonate, are added; paraformaldehyde is sensitive to strong oxidizing and reducing agents.
Solubility	soluble in water and polar solvents; the solubility of the polymerisates decreases with increasing degree of polymerisation
Toxicity data	
LD ₅₀ oral	800 mg/kg rat
LC ₅₀ inhalative (exposure 4 h)	1070 mg/m ³ for rats
LD ₅₀ dermal	> 2000 mg/kg rabbit
Severely irritant to the skin and mucous membranes - sensitisation may occur. Formaldehyde is regarded as possibly carcinogenic to humans. However before rating this statement one should have a look to a critical review with regard to formaldehyde and cancer by McLaughlin (1994) who states: “When the epidemiologic data on formaldehyde and human cancer are examined in light of the widely accepted causal criteria of strength of the association consistency and specificity of results, dose-response effect, and biologic coherence and plausibility, the studies published so far fail to provide credible causal evidence.”	
Exposure limit (occupational)	Germany 0.6 (0.5) mg/m ³ (ppm)
Chemical name	2.1b. 1,3,5-Trioxane
Chemical formula	C ₃ H ₆ O ₃
Structural formula	
Molecular mass	90.08
CAS-No.	110-88-3
EC-No.	203-812-5
Synonym/common name	trioxymethylene, Triformol
Supplier	SIGMA ALDRICH
Chemical and physical properties	
Appearance	trioxane is the cyclic trimer of formaldehyde which originates from gaseous formaldehyde in the form of volatile needles
Content %	≥99
Boiling point/range °C (101 kPa)	112–115
Melting point °C	59–62

Flash point °C	45
Auto ignition temperature °C	414
Stability	decomposition under the influence of acids, dampness and strong oxidizing agents
Solubility	soluble in water and polar solvents
Chemical name	2.1c. Formaldehyde solution stab.
Chemical formula	CH ₂ O
Structural formula	O = CH ₂ · H ₂ O; HO-CH ₂ -(O-CH ₂) _n -OH
Molecular mass	30.03
CAS-No.	50-00-0
EC-No.	200-001-8; EEC-no. 5
Synonym/common name	Formalin
Supplier	BASF, BAYER, HOUGHTON CHEMICAL CORP.
Chemical and physical properties	
Appearance	the introduction of formaldehyde into water leads to Formalin containing 30–45% by weight formaldehyde; the 37% standard is characterized as follows: clear, colourless liquid with the pungent odour of formaldehyde 37; contains 10–15° methanol to avoid polymerization
Content %	37
Density g/ml (20°C)	1.083
Refractive index nD (20°C)	1.3765
Flash point °C	56
Stability	sensitive to strong oxidizing and reducing agents; reacts readily with amines, proteins, phenols
Solubility	miscible with water and alcohols
Toxicity data	
LD ₅₀ subcutaneous	300 mg CH ₂ O/kg mouse

Formalin is a strong irritant agent for the skin and mucous membranes. However, the presence of out-gassing formaldehyde is already registered at concentrations of approx. 0.1–1.0 ppm by the very sensitive sense of smell. Further data see 2.1a. Paraformaldehyde.

Antimicrobial effectiveness/applications

The powerful antimicrobial properties of formaldehyde were first demonstrated in 1886 (Loew).

Looking at the MIC in Table 10 more closely it becomes obvious that bacteria are better covered by the antimicrobial efficacy of formaldehyde than fungi and yeasts. In addition, formaldehyde also has sporicidal and virucidal effects. This extremely broad range of effectiveness is a result of the distinct chemical reactivity of the formaldehyde which, as already mentioned before, belongs to the electrophilic active agents. When using formaldehyde, e.g. as a preservation agent, this reactivity may naturally also have an adverse effect. The coincidence of formaldehyde with ammonia thus leads to an inactivation due to the formation of hexamethylenetetramine (3.3.1.). The reaction with proteins also results in inactivation; at the same time disturbing coagulates are being formed by this reaction. On the other hand, formaldehyde is – without loss in effectiveness – compatible with anionic, cationic and non-ionic detergents and effective – largely independent of the pH value (optimum pH range: 3–10).

Formalin and paraformaldehyde are significant forms for applications which require preservatives with a high degree of purity and colourlessness. When evaluating formaldehyde as a preserving agent its reactivity and volatility are considered a disadvantage, because both impair permanence and compatibility and are moreover responsible for odour nuisances. The volatility is on the other hand a benefit by converting formalin and paraformaldehyde into preservatives with vapour phase effect, e.g. in the head space of cans and tanks. The multipurpose microbicide is also attractive from the economic point of view and still used as an active ingredient in disinfectants and preservatives.

As a preservative for industrial fluids, formaldehyde (formalin and paraformaldehyde) is preferably used in cosmetic products and in polymer dispersions (natural and synthetic latex) often in combination with other microbicides. Formaldehyde is listed in the EC list of preservatives allowed for the in-can protection of cosmetics (maximum authorized concentration: 0.2%, except for products for oral hygiene: 0.1%, prohibited in aerosol dispensers). Percentage of use in US cosmetic formulations: 0.11% paraformaldehyde. Oil, fat and wax emulsions, starch and dextrine glues, adhesive dispersions, pigment and filler slurries, thickening solutions and other aqueous formulations containing no proteins can be preserved by the addition of formaldehyde, too: in order to

Table 10 Minimum inhibition concentrations (MIC) of formaldehyde in nutrient agar

Test organism	MIC (mg/litre)
Fungi	
<i>Aspergillus niger</i>	300
<i>Aureobasidium pullulans</i>	140
<i>Chaetomium globosum</i>	70
<i>Penicillium glaucum</i>	750
<i>Rhizopus nigricans</i>	200
<i>Trichoderma viride</i>	1200
Yeasts	
<i>Candida albicans</i>	1000
<i>Candida crusei</i>	200
<i>Torula rubra</i>	700
<i>Torula utilis</i>	80
Bacteria	
<i>Aerobacter aerogenes</i>	50
<i>Aeromonas punctata</i>	15
<i>Bacillus subtilis</i>	15
<i>Desulfovibrio desulfuricans</i>	15
<i>Escherichia coli</i>	45
<i>Proteus vulgaris</i>	10
<i>Pseudomonas aeruginosa</i>	60
<i>Pseudomonas fluorescens</i>	75
<i>Staphylococcus aureus</i>	20
Formaldehyde resistant bacteria ^a	1400

^a Paulus (1976).

improve the permanence and the range of effectiveness (synergism) generally a further microbicide, preferably a fungicide, is also applied.

Formalin or paraformaldehyde can also be employed as vapour phase microbicides (fungicides) without residues being left (e.g. in packings).

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.2. Acetaldehyde
Chemical formula	C ₂ H ₄ O
Structural formula	CH ₃ -CHO
Molecular mass	44.05
CAS-No.	75-07-0
EC-No.	200-836-8
Synonym/common name	ethanal, ethylaldehyde
Supplier	SIGMA ALDRICH
Chemical and physical properties	
Appearance	colourless, inflammable, highly reactive fluid of low viscosity, characterized by a pungent, narcotic odour
Content%	> 99.5
Boiling point/range °C (101 kPa)	21
Solidification point °C	-125
Density g/ml (20°C)	0.785
Vapour density g/l (20°C)	1.52
Refractive index n _D (20°C)	1.3316
Flash point °C	-40
Upper flammability limit %v/v i. air	60
Lower flammability limit %v/v i. air	4
Stability	pyrolyzes to CO ₂ and methane; sensitive to air and oxidizing agents; the reactivity of acetaldehyde manifests itself in numerous addition and condensation reactions; it tends to polymerize to paraldehyde (cyclo-trimeric) and metaldehyde (cyclo-tetrameric); the latter is used as a molluscicide

Solubility	highly soluble in H ₂ O and organic solvents	
Toxicity data		
LD ₅₀ oral	661 mg/kg rat	900 mg/kg mouse
LD ₅₀ subcutaneous	640 mg/kg rat	
LD ₅₀ intraperitoneal	500 mg/kg mouse	
LD ₅₀ inhalative (4 h) for rats	13300 mg/m ³	
Irritant to the skin and mucous membranes; photosensitizer.		
There are reasons for the suspect that acetaldehyde acts cancerogenic.		
Exposure limits (occupational)	France	180 (100) mg/m ³ (ppm)
	Germany	90 (50) mg/m ³ (ppm)
	UK	37 (20) mg/m ³ (ppm)

Antimicrobial effectiveness/application

Although acetaldehyde itself is not applicated as a microbicide, it is described here because of the fact that 2,6-dimethyl-1,3-dioxane-4-ol, the unstable addition product of 3 mol acetaldehyde, may be stabilized by acetylation to 2,6-dimethyl-1,3-dioxane-4-yl-acetate, an acetaldehyde releasing compound exhibiting a broad spectrum of antimicrobial efficacy which is used as a preservative for aqueous functional fluids (see 4.1.).

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.3. Glyoxal
Chemical formula	C ₂ H ₂ O ₂
Structural formula	OHC-CHO
Molecular mass	58.04
CAS-No.	107-122-2
EC-No.	203-474-9
Synonym/common name	ethanedial, oxaldehyde
Supplier	BASF, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to yellow prisms or fluid; in general the lowest dialdehyde is available as a 40% solution (8.8 M) in water (density at 20°C 1.27 g/ml; nD (20°C) 1.409)
Content%	> 99
Boiling point/range °C (101 kPa)	51
Melting point °C	15
Density g/ml (20°C)	1.14
Refractive index nD (20°C)	1.3826
Stability	tends to polymerize; sensitive to strong bases and oxidizing agents; the reactivity of glyoxal is comparable with that of formaldehyde
Solubility	> 40% in water, soluble in alcohols
Toxicity data for a 40% solution of glyoxal in water	
LD ₅₀ oral	> 2000 mg/kg rat
LD ₅₀ dermal	> 2000 mg/kg rat
Irritant to skin and mucous membranes.	
Ecotoxicity (source BASF Specialty Chemicals)	
EC ₁₀ for bacteria (16 h)	10–100 mg/l
LC ₅₀ for <i>Leuciscus idus</i> (96 h)	> 100 mg/l
EC ₅₀ for <i>Daphniae</i> , acute (48 h)	> 100 mg/l

Antimicrobial effectiveness/application

Glyoxal is effective against a broad range of micro-organisms including gram-positive and gram-negative bacterial, fungi, spores and certain viri. The minimum inhibition concentrations listed in Table 11 demonstrate the microbistatic efficacy of glyoxal. A microbicidal effect exhibits glyoxal at relatively high concentrations (1–4%) only. Nevertheless it is suitable for the application in disinfectants and sanitizers for hospitals and animal husbandry uses, generally in combination with other active ingredients to improve performance.

Table 11 Minimum inhibition concentrations (MIC.) of a 40% solution of glyoxal in H₂O (Source: BASF Specialty Chemicals.)

Organism	MIC (%)	
<i>Staphylococcus aureus</i>	ATCC 6538	0.25
<i>Escherichia coli</i>	ATCC 11229	0.50
<i>Proteus mirabilis</i>	ATCC 14153	0.50
<i>Pseudomonas aeruginosa</i>	ATCC 15442	0.50
<i>Candida albicans</i>	ATCC 10231	2.50

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.4. Succinaldehyde
Chemical formula	C ₄ H ₆ O ₂
Structural formula	OHC-CH ₂ -CH ₂ -CHO
Molecular mass	86.09
CAS-No.	638-37-9
EC-No.	211-333-8
Synonym/common name	butane-1,4-dial
Supplier	BASF

For further information see: 5. Succinaldehyde releasing compound

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.5. Glutaraldehyde
Chemical formula	C ₅ H ₈ O ₂
Structural formula	OHC-(CH ₂) ₃ -CHO
Molecular mass	100.12
CAS-No.	111-30-8
EC-No.	203-856-5; EEC-no. 48
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	pentane-1,5-dial, 1,5-pentanedione, glutaric dialdehyde, Glutaral
Supplier	BASF, BAYER, DOW
Chemical and physical properties	
Appearance	oily fluid; b.p. (101 kPa) 187–189°C (decomposition), 71–72°C at 1,3 kPa; soluble in water
Content% i. H ₂ O	25 50
Boiling point/range °C (101 kPa)	101
Solidification point °C	–10 –21
Density g/ml (20°C)	1.066 1.131
Vapour pressure hPa (20°C)	21.86 20
Surface tension mN/m (20°C)	45 48
Refractive index nD (20°C)	1.375 1.421
pH at 25°C	3.1–4.5 3.1–4.5
Stability	volatile with water vapour; tends to polymerize in water; stable in acidic solutions; only stable to a limited extent in alkaline solutions; inactivation by ammonia and primary amines at neutral and higher pH values; sensitive to strong oxidation agents

Solubility	complete in water, lower alcohols and glycols	
Toxicity data (for a 50% solution)		
LD ₅₀ oral	320 mg/kg rat; 134 mg/kg rat	
dermal	> 2600 mg/kg rabbit	
intraperitoneal	17.9 mg/kg rat	
intravenous	9.8 mg/kg rat	
LC ₅₀ inhalation (4 h)	480 mg/m ³ for rats	
Irritant to skin and mucous membranes; sensitization possible. Non-mutagenic.		
Exposure limits	France	0.8 (0.2) mg/m ³ (ppm
	Germany	0.4 (0.1) mg/m ³ (ppm)
	UK	0.2 (0.05) mg/m ³ (ppm)
Ecotoxicity (source: BASF Specialty Chemicals)		
EC/LC10 for bacteria (17 h)	1–10 mg/l	
EC/LC50 for <i>Leuciscus idus</i> (96 h)	10–100 mg/l	
EC/LC50 for <i>Daphniae</i> , acute (48 h)	10–100 mg/l	

Antimicrobial effectiveness/applications

Glutaraldehyde was introduced as an antimicrobial substance not before 1962 and has been studied and reviewed extensively in the meantime (Gorman et al., 1980).

The MIC's listed in Table 12 demonstrate the broad activity spectrum of glutaraldehyde.

Glutaraldehyde may be considered as a "chemosterilizer" because of its capability to destroy bacteria, fungi and the corresponding spores, tubercle bacilli and viri, although non-lipid viri usually are more resistant to the attack of glutaraldehyde than the enveloped lipophilic viri. The most important property of glutaraldehyde is, however, its sporicidal efficacy, as there are only a few sporicides available. Glutaraldehyde is at a more acceptable contact time 2–8 times more sporicidal than formaldehyde which has additionally several other disadvantages including irritation and lack of penetration. Power et al. (1989) investigated the uptake to bacterial spores, germinating and outgrowing, and vegetative cells of sporing and non sporing bacteria and found, that germinated and outgrowing spores absorbed more glutaraldehyde than resting spores, but less than vegetative cells which were completely killed within 10 min by 2% (w/v) alkaline glutaraldehyde, whereas bacterial spores were only inactivated after a matter of hours. Low concentrations of alkaline and acid glutaraldehyde increased the surface hydrophobicity and inhibited the germination of bacterial spores, the alkaline solution to a greater extent in both cases. Beside the rapidity of action (see Table 13) – like bacteria most viri, too are destroyed within 10 min – the activity in the presence of organic matter (e.g. serum) and the ease of use are further advantages of glutaraldehyde.

Table 12 Minimum inhibition concentrations (MIC) of glutaraldehyde (source: BASF Specialty Chemicals)

Organism	MIC (ppm)	
Gram positive bacteria		
<i>Staphylococcus aureus</i>	NCIB 9518	50
<i>Bacillus subtilis</i>	NCTC 10073	1250
<i>Bacillus cereus</i>	NCOO 2599	1250
Gram negative bacteria		
<i>Legionella pneumophila</i>	(Industrial isolate)	1250
<i>Klebsiella aerogenes</i>	NCTC 418	150
<i>Klebsiella pneumoniae</i>	PC 1602	150
<i>Pseudomonas aeruginosa</i>	NCIMB 8626	150
<i>Pseudomonas fluorescens</i>	NCIB 9046	150
Sulphate reducing bacteria		
<i>Desulphovibrio desulphuricans</i>	NCIB 8301	60
Yeasts		
<i>Candida albicans</i>	NCPF 3179	1250
<i>Saccharomyces cerevisiae</i>	NCTC 87	1250
Moulds		
<i>Stachybotrys atra</i>	IMI 82021	250
<i>Penicillium funiculosum</i>	IMI 87160	250
<i>Aspergillus niger</i>	IMI 14007	500

Table 13 Minimum microbicidal concentration (%) of glutaraldehyde (50%) (with and without soiling –0.2% albumin –) at contact time: (source: THOR Biocides Division)

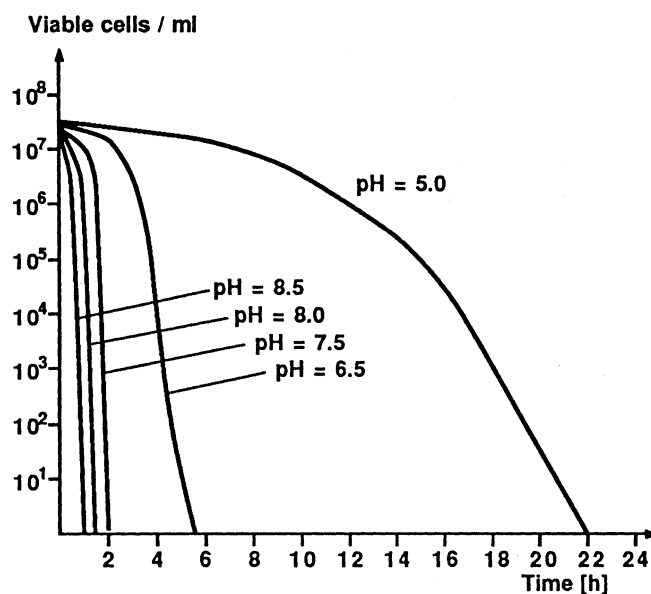
	5 minutes	30 minutes	60 minutes
Without soiling:			
<i>Staphylococcus aureus</i>	0.10	0.025	0.0125
<i>Pseudomonas aeruginosa</i>	0.10	0.050	0.025
<i>Candida albicans</i>	1.0	0.750	0.750
With soiling:			
<i>Staphylococcus aureus</i>	0.10	0.050	0.025
<i>Pseudomonas aeruginosa</i>	0.10	0.050	0.025
<i>Candida albicans</i>	1.50	1.0	0.750

The mechanism of microbicidal action of glutaraldehyde is based on its two toxophoric (aldehyde) groups which can interact with nucleophilic microbial cell constituents, e.g. amino and thiol groups of proteins, with ring nitrogen atoms of purine bases. This interaction increases with increasing pH. Thus pH is the most important factor in regulating the activity of glutaraldehyde under typical use conditions. The curves in Figure 3 show that the rate of kill of the gram-negative bacterium *Escherichia coli* is approximately twenty times faster at pH 8.5 than pH 5. One can assume that in an acidic medium the reactive sites of the cell wall are protonated and therefore protected from an interaction with the toxophoric groups of glutaraldehyde. This explains also the fact, that in the presence of ammonia, primary amines or protein, which can interact with (inactivate) glutaraldehyde, the latter is significantly effective only at pH 4.5 or less. In the acidic state the effectiveness of glutaraldehyde is presumably caused by the ability of the active ingredient to penetrate the cell wall and to reach internal areas of neutral pH which allows interaction with free internal amino groups; however, the rate of kill is slower (s.a.). In the acidic state, the addition of surfactants permit faster penetration of glutaraldehyde through the cell wall thus increasing the activity. Suitable surfactants for the combination with glutaraldehyde may be cationic, nonionic or anionic agents.

Glutaraldehyde is usually obtained commercially as 2% or 25% or 50% solution of acidic pH. The degree of polymerization is negligible in acidic solution but high at basic pH. Polymerization leads to an extensive loss of aldehyde groups and in consequence to a loss of effectiveness, however, in weeks whereas the microbicidal efficacy is observed within minutes or hours, especially in basic media. Glutaraldehyde therefore is applied more as a disinfecting and sterilizing agent than as a preservative.

Optimum performance involves balancing factors such as pH, temperature matrix and additives in the final product. In practice glutaraldehyde is generally available as a 2% solution to which an activator is added to bring the pH to approximately 8 before application, e.g. disinfection of instruments. The activated solution disinfects at room temperature within 10 min and sterilizes within 10 h. The activated solution has to be discarded 14 days after activation. Another important application for glutaraldehyde solution is the disinfection of surfaces.

Because of its effectiveness against slime forming microbes (minimum inhibition concentration approx. 2–5 mg/litre) glutaraldehyde may be used in paper mills as a slimicide which does not cause any waste water

**Figure 3** Rate of kill curves for glutaraldehyde (conc. 44 ppm) at different pH values; temperature 20°C; test organism *Escherichia coli* (source: Union Carbide Corporation Specialty Chemicals Division, USA).

problems. Especially since paper mills increase the pH in their process water to 7 or even to the slightly alkaline state, they profit from the higher activity of glutaraldehyde in such media. – Oil recovery is another significant application field for glutaraldehyde. See Part I, chapter 5.4.

At concentrations of 0.05–0.2% glutaraldehyde can act as a preservative for industrial fluids, the pH of which is between 4 and 6 (optimum pH 5). The average lifespan for glutaraldehyde is 6 month at pH 7, however, up to 2 years at pH 4.

In the EC glutaraldehyde is listed on the Cosmetics Directive; max. addition rate: 0.1%. The warning “contains glutaraldehyde” must appear on labels when concentrations in the finished product exceed 0.05%. Not allowed in sprays. Percentage of use in US cosmetic formulations: 0.20%.

In North America glutaraldehyde has received the clearance for use as

- a food contact adhesive preservative
- a paper slimicide (max. 750 g a.i./ton of paper)
- a preservative for pigments and filler slurries for paper and paper board for aqueous and fatty food (max. 300 ppm based on solids)
- a component of process aids in sugar manufacture.

As glutaraldehyde is compatible with most surfactants (non-ionic, anionic, cationic) it may advantageously be used in corresponding formulations together with other microbicides, like phenolics such as 2-phenyl-phenol (7.4.1.), 4-chloro-3-methyl-phenol (7.3.1.), 2-benzyl-4-chloro-phenol (7.3.5.), 4-chloro-3,5-dimethyl-phenol (7.3.2.), or like quaternary ammonium compounds (18.1).

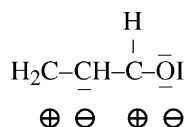
Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.6. 2-Propenal
Chemical formula	C_3H_4O
Structural formula	$CH_2 = CH-CHO$
Molecular mass	56.6
CAS-No.	107-02-8
EC-No.	203-453-4
EPA/FIFRA 1988	Pesticide subject to registration or re-registration
Synonym/common name	acrolein, acrylaldehyde, acrylic aldehyde, allyl aldehyde, ethylene aldehyde
Supplier	BAKER PETROLITE, DEGUSSA, SIGMA ALDRICH
Chemical and physical properties	
Appearance	colourless fluid with a pungent and severely irritant odour
Content %	~97 (stab. with 0.2% hydrochinone and ~3% H_2O)
Boiling point/range °C (101 kPa)	53 (decomposition)
Melting point °C	–87
Density g/ml (20°C)	0.839
Vapour pressure hPa (20°C)	296
Vapour density g/l	194
Refractive index nD (20°C)	1.4017
Flash point °C	–18.9
Auto ignition temperature °C	240
Upper flammability limit %v/v i.air	31
Lower flammability limit %v/v i.air	2.8
Stability	tends to polymerize if not stabilized; reacts with numerous compounds; half-life in H_2O : 150 h at pH 5; 120–180 h at pH 7; 5–40 h at pH 9
Solubility g/l (20°C)	267 i. H_2O , complete in lower alcohols, ketones, benzene, diethyl ether, and other common organic solvents
Toxicity data	
LD ₅₀ oral	26 mg/kg rat
Intraperitoneal	4 mg/kg rat
subcutaneous	50 mg/kg rat
dermal	200 mg/kg rabbit
LC ₅₀ inhalation (4 h)	18 mg/m ³ for rats

Powerfully irritant to the skin;
 lachrymatory effect and severely irritant action on respiratory organs (2.3 mg/m³ are already intolerable);
 2 mg (24 h) cause severe skin irritation (rabbit skin);
 50 µg (24 h) cause severe eye irritation (rabbit eye).

Exposure limits (occupational)	France 0.25 (0.1) mg/m ³ (ppm) Germany 0.25 (0.1) mg/m ³ (ppm) UK 0.23 mg/m ³
Ecotoxicity	
LC ₁₀₀ for fish	approx. 1–5 mg/l

Antimicrobial effectiveness/application

The two toxophoric groups in the acrolein molecule, namely the activated vinyl group and the aldehyde group, equip the molecule with two electrophilic carbon atoms C-1 and C-3 (see Part I, Chapter 2):



Both C-atoms are able to attract nucleophilic compounds. Thiols (R-SH) for instance link preferably to C-1, forming β-mercapto-propionaldehyde (R-S-CH₂-CH₂-CHO). Sodium hydrogensulfite occupies not only C-1, but also C-2. Apparently acrolein is highly reactive and accordingly highly effective and may be defined as a biocide rather than a microbicide. hydrogensulfite occupies not only C-1, but also C-2.
 Minimum inhibition concentrations of acrolein for:

Slime forming organisms	1 mg/l
Fresh water algae	5–10 mg/l

Acrolein may be used as a slimicide and algicide in industrial water circuits and injection water for oil recovery as a non-persistent active ingredient which does not cause waste water problems. It was also recommended for combatting submerging aquatic plants (Austin, 1964). However, because of its powerful irritant action and toxicity, handling of acrolein is very difficult; therefore its use has been restricted in favour of safe compounds which in-situ generate acrolein (Werle et al., 2000); see 6.1.

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.7. Chloroacetaldehyde
Chemical formula	C ₂ H ₃ ClO
Structural formula	Cl-CH ₂ -CHO
Molecular mass	78.50
CAS-No.	107-20-0
EC-No.	203-472-8
EPA-TSCATS	data base, Jan. 2001
Synonym/common name	monochloroacetaldehyde, 2-chloro-1-ethanal
Supplier	WACKER-CHEMIE
Chemical and physical properties	
Appearance	colourless, volatile fluid (boiling point 85°C at 101 kPa) with a pungent odour
Content %	45 in water
Boiling point/range °C (101 kPa)	80–100
Density g/ml (20°C)	1.236
Flash point °C	53.3

Stability	the addition of NaHSO ₃ causes precipitation of chloroacetaldehyde sodium hydrogen sulfite, a colourless, nearly odourless powder, Cl-CH ₂ -CH-OH which in aqueous solutions liberates SO_3Na chloroacetaldehyde
Solubility	complete in water, soluble in usual organic solvents
Toxicity data	
LD ₅₀ oral	89 mg/kg rat; 82 mg/kg mouse
LD ₅₀ intraperitoneal	7 mg/kg mouse or rat 2.5 mg/kg guinea pig
LC ₅₀ inhalation (1 h) for rats	650 mg/m ³
LD ₅₀ dermal	267 mg/kg rabbit
Severely irritant (corrosive) to the skin and mucous membranes. Possibly carcinogenic and genotoxic.	
Exposure limits (occupational)	France/Germany 3 (1) mg/m ³ (ppm) United Kingdom 3.3 (1) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

Chloroacetaldehyde exhibits bactericidal and fungicidal efficacy. Mainly in use is the addition product of sodium hydrogensulfite and chloroacetaldehyde, as it is easily to handle and nearly odourless. Worth mentioning is its use in ground disinfectants.

Microbicide group (substance class)	2. ALDEHYDES
Chemical name	2.8 alpha-Bromocinnamaldehyde (BCA)
Chemical formula	C ₉ H ₇ BrO
Structural formula	C ₆ H ₅ -CH = CBr = CHO
Molecular mass	211.06
CAS-No.	5443-49-2
EC-No.	266-637-6
Synonym/common name	2-bromo-3-phenyl-2-propenal, Alphabrocine, BCA
Supplier	ABCR, SIGMA-ALDRICH, WUXIMENHUA CHEMICAL ENGINEERING CO. LTD.
Chemical and physical properties	
Appearance	pale yellow powder with an odour similar to cinnamon
Content	~99
Melting point °C	71–72
Bulk density g/l (20°C)	650–700
Vapour pressure hPa (50°C)	0.0013
Stability	stable in a pH range of 3–11; decomposition at 230–240°C; although BCA represents an unsaturated aldehyde like acrolein (2.6.) and additionally disposes of a halogen atom in α-position to the carbonyl group, its electrophilic character (reactivity) is not as distinct as that of acrolein, since the phenyl group of BCA ensures a resonance stabilized system (see Part I, Chapter 2); however the alpha halogen substituent strengthens the electrophilicity of the beta carbon atom, thus promoting nucleophilic addition reactions at the double carbon bond
Solubility	sparingly soluble in water, soluble in organic solvents such as ethanol, acetone, methylethylketone, benzene, toluene, xylene

Toxicity data

LD ₅₀ oral	1450 mg/kg rat 470 mg/kg mouse
LD ₅₀ intraperitoneal	513 mg/kg rat 822 mg/kg mouse
LD ₅₀ subcutane	1210 mg/kg rat 2200 mg/kg mouse

Irritant to skin and mucosa.

Mutagenic to *Salmonella typhimurium* strain TA100.

Antimicrobial effectiveness/applications

BCA is a volatile chemical which may be used as a vapour phase microbicide. Small amounts of it are able to inhibit the growth of mould producing fungi, of yeasts and bacteria. In containers, machines, parts of telecommunication devices BCA is used as a vapour phase microbicide preventing mould growth during storage and transport of the devices. It is especially useful where articles do not allow the incorporation of a microbicide for protection against biodeterioration.

Table 14 Minimum inhibition concentrations (MIC) of BCA in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	50
<i>Aspergillus niger</i>	100
<i>Aureobasidium pullulans</i>	100
<i>Chaetomium globosum</i>	50
<i>Lentinus tigrinus</i>	5
<i>Penicillium glaucum</i>	35
<i>Sclerophoma pityophila</i>	100
<i>Trichoderma viride</i>	100
<i>Escherichia coli</i>	35
<i>Pseudomonas aeruginosa</i>	500
<i>Staphylococcus aureus</i>	35

Microbicide group (substance class)

Chemical name

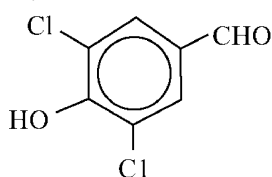
Chemical formula

Structural formula

2. ALDEHYDES

2.9. 3,5-Dichloro-4-hydroxy-benzaldehyde (DCHB)

C₇H₄Cl₂O₂



Molecular mass

191.02

CAS-NO.

2314-36-5

EC-No.

unknown

Synonym/common name

1-formyl-3,5-dichloro-4-hydroxy-benzene, (3,5-dichloro-4-hydroxy)phenyl-methanal

Supplier

Chemical and physical properties

Appearance

crystalline solid with a characteristic odour

Content %

~99

Melting point °C

156

Solubility

highly soluble in ethanol, propylene glycol, ether, acetic acid, less soluble in benzene, chloroform, sparingly soluble in water

Toxicity data

LD ₅₀ oral	> 5 g/kg rat
dermal	> 2 g/kg rat (exposure: 24 h)

Not irritant to skin and mucous membranes. Ames test negative.

Antimicrobial effectiveness/application

Because of its broad spectrum of activity which covers a wide range of bacteria, yeasts and moulds, DCHB may be a suitable preservative for the in-can protection of industrial fluids, e.g. detergent concentrates and surfactant-based products; addition rates: 0.1–0.15%.

Chelating agents, such as EDTA, support the efficacy of DCHB so that lower concentrations can be applied, e.g. 0.05–0.1% (Maddox, 1988). Assuming that further toxicological evaluations will deliver satisfactory results, DCHB can be used as a preservative in cosmetics.

Table 15 Minimum inhibition concentrations (MIC) of a 30% solution of DCHB in propylene glycol according to Maddox (1988)

Test organism	MIC (mg/litre)
<i>Pseudomonas aeruginosa</i> -isolate	1500
<i>Pseudomonas putida</i> -isolate	1500
<i>Pseudomonas fluorescens</i> -isolate	700
<i>Pseudomonas fluorescens</i>	70
<i>Klebsiella oxytoca</i> -isolate	700
<i>Klebsiella pneumoniae</i> -isolate	1500
<i>Proteus vulgaris</i>	700
<i>Citrobacter freundii</i> -isolate	150
<i>Salmonella enteritidis</i>	700
<i>Escherichia coli</i> -isolate	700
<i>Staphylococcus aureus</i>	700
<i>Streptococcus aureus</i>	1500
<i>Staphylococcus epidermidis</i>	1500
<i>Candida albicans</i>	2300
<i>Saccharomyces cerevisiae</i>	2300
<i>Penicillium expansum</i>	2300
<i>Penicillium notatum</i>	1500
<i>Cladosporium herbarum</i>	700

Microbicide group (substance class)

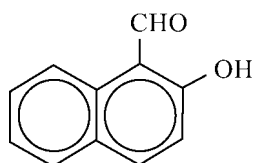
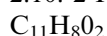
Chemical name

Chemical formula

Structural formula

2. ALDEHYDES

2.10. 2-Hydroxy-1-naphthaldehyde (HNA)



Molecular mass

172.19

CAS-No.

708-06-5

EC-NO.

211-902-0

EPA-Reg./TSCA

Section 8(B) Chemical Inventory

Synonym/common name

1-formyl-2-hydroxy-naphthalene, (2-hydroxy)naphthyl-methanal

Supplier

SIGMA ALDRICH

Chemical and physical properties

Appearance

crystalline solid

Content %

~99

Boiling point/range °C (3.6 kPa)

192

Melting point °C

82–85

Solubility

practically insoluble in water, soluble in alcohol, ether, petroleum ether, highly soluble in alkaline solutions

Toxicity data

LD₅₀ oral > 5 g/kg rat
 intraperitoneal > 710 mg/kg rat

Irritant to skin and mucosa.

Antimicrobial effectiveness/application

HNA is especially effective against mould producing fungi; it offers an extraordinary broad and equal spectrum of activity. Sharma (1989) has determined the minimum inhibition concentrations for 40 different species of fungi and found that none of the species tolerated more than 60 mg/litre of HNA. At the inhibitory concentrations of HNA the secretion of extracellular enzymes (amylase, cellulase, protease lipase) is suppressed (=100% inhibition). According to the findings of Sharma (1989) the morphological changes of test organism *Aspergillus flavus* were comparable to those produced by chlorinated phenols, e.g. p-chloro-m-cresol (7.3.1.), demonstrating that HNA belongs to the membrane-active substances. The presence of the aldehyde groups makes HNA, in addition, an electrophilic agent and increases its toxicity.

Because of its broad spectrum of effectiveness, its insolubility in water and its stability it is proposed to use HNA as a long-term protectant against biodeterioration of materials, e.g. leather, footwear, cotton, textiles, paper.

Microbicide group (substance class

Chemical name

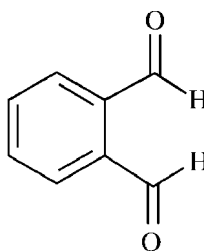
Chemical formula

Structural formula

2 ALDEHYDES

2.11. Phthalic dialdehyde

C₈H₆O₂



Molecular mass

134.14

CAS-No.

643-79-8

EC-No.

211-402-2

EPA TSCA

Section 8 (B) Chemical Inventory

Synonym/common name

OPA, ortho-phthalaldehyde, benzene 1,2-dicarbaldehyde

Supplier

JOHNSON & JOHNSON

Chemical and physical properties

Appearance

nearly colourless crystalls

Content (%)

~99

Melting point °C

54–56

Stability

stable under normal conditions

Solubility g/l (25°C)

50 in H₂O, soluble in methanol, ethanol, ethylene glycol, tetrahydrofuran

Toxicity data

Not yet fully examined. Irritant to skin and mucosa.

Antimicrobial effectiveness/applications

Theis and Leder (1993) demonstrated in aerobic biofilm experiments with an oil field isolate of aerobic bacteria, containing predominantly *Pseudomonas* species, that OPA is more effective than formaldehyde (FA, 2.1a.) or glutaraldehyde (GA, 2.5.) in killing or inhibiting the growth of sessile microorganisms (see Table 16). OPA is effective without activation and is able to inactivate GA-resistant strains of *Mycobacterium chelonae*. On the other hand OPA unlike GA acts not sporicidal at its in-use concentration of 0.5% (w/v) and normal pH (6.5.).

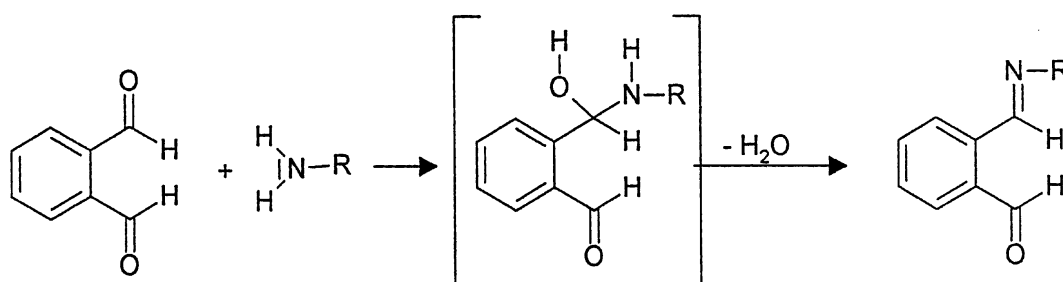
With regard to a proposed mechanism of action of OPA Simons et al. (2000) interpret the observation that OPA is a less potent crosslinking agent than GA as an influence of steric restrictions of the aromatic dialdehyde in contrast to GA which is composed of a flexible aliphatic chain. In addition the two carbonyl groups of OPA are part of a resonance stabilized system and consequently are less reactive than aliphatic aldehydes such as GA or FA in nucleophilic addition reactions. The fact that OPA in contrast to other aldehydes reacts only with

Table 16 Reduction of cfu in a culture of aerobic bacteria (oil field isolate) after 1 and 4 h exposure to different aldehydes

Aldehyde	Conc. ppm*	-log reduction after-	
		1 h	4 h
GA	10	0.1	0.7
GA	25	0.3	1.7
GA	50	5.1	5.1**
OPA	5	0.8	5.1
OPA	10	1.4	5.1
OPA	25	5.1	5.1
FA	250	2.0	5.1
FA	500	2.6	5.1
FA	1000	5.1	5.1

*All concentrations are ppm by weight active ingredient

**5.1 represents complete kill

**Figure 4** Nucleophilic addition of a primary amino group to orthophthalaldehyde followed by elimination of H₂O.

primary amines, or peptides containing such amino groups, is also conditioned by the tendency of the molecule to reinstall the resonance stabilized system immediately. That is not possible if a secondary amine is added nucleophilically (see Figure 4). However these properties of OPA which reduce its antimicrobial efficacy are more than compensated by the lipophilic aromatic nature of the dialdehyde which apparently assists its uptake through the outer layers of mycobacteria and Gram-negative bacteria.

It is recommended to use OPA for controlling biofouling caused by sessile and planctonic micro-organisms in industrial water systems, e.g. for pulp and paper manufacture and for oil field water flooding. Normal addition rates move between 10 to 250 ppm. A synergistic slime control effect is achieved, if a combination of bis [tetra-kis(hydroxymethyl)phosphonium] sulfate (3.6) and OPA is introduced into white water.

3 Formaldehyde releasing compounds

Formaldehyde as such is often too volatile and too reactive to be used as a microbicide for the protection of materials or in disinfectants. It additionally produces unwelcome side-effects, and has an insufficient balanced range of activity. One therefore has looked for formaldehyde releasing compounds which do not exhibit the disadvantageous formaldehyde effects but maintain or even improve the antimicrobial action of formaldehyde.

The first step towards formaldehyde releasing compounds generally consists in the process of hydroxymethylation, that is, introduction of the hydroxymethyl group into such molecules as possess active hydrogen atoms which allow a reversible reaction with formaldehyde, the series ranging from hydrol to the amino acid taurine (Figure 5).

The range of variety is an enormous one, and it is broadened even more by the ability of the hydroxymethyl compounds to react as well (Figure 6).

Formaldehyde releasing compounds can be solids or liquids, water soluble or oil soluble, odourless, alkaline, neutral, or slightly acidic. They open up applications to the active agent, formaldehyde, which would otherwise be closed to it due to its unfavourable properties.

3.1 *O*-Hydroxymethyl compounds (hemiformals) and formals

The reaction of formaldehyde with alcohols (R-OH), easily taking place under neutral or weakly alkaline conditions, leads to the formation of hemiformals which are relatively heat resistant and in equilibrium with

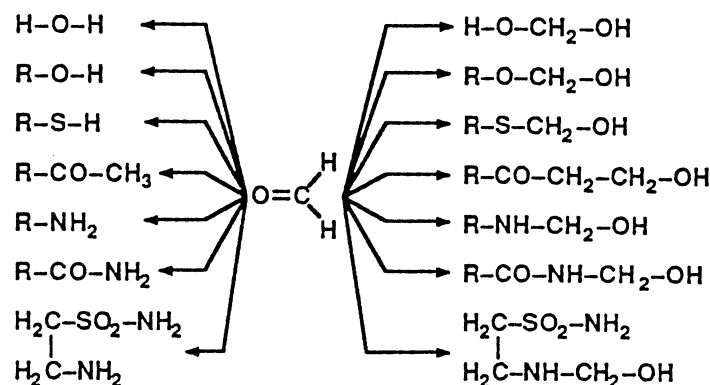


Figure 5 The process of hydroxymethylation – reaction of formaldehyde with active hydrogen atoms.

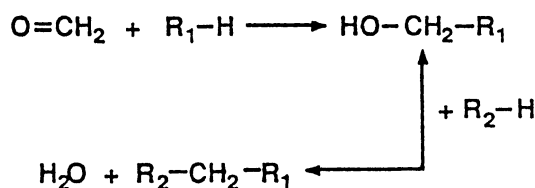


Figure 6 Reversible formation of hydroxymethyl/methylene compounds.

the starting products. However, the equilibrium is widely shifted to the side of the hemiformal; free formaldehyde is detectable in mere traces. Under acidic conditions the reaction goes on to produce formals.

If formaldehyde acts upon the more acidic phenolic hydroxy group, the reaction products obtained are unstable and cannot be isolated as the equilibrium is widely shifted to the side of the starting products. However, for practical applications one takes advantage of the antimicrobial activities of such mixtures which are based on the effective spectra of the mixing partners usually complementing each other advantageously; a remarkable synergism is often also noticed. One has to keep in mind, however, that under certain conditions, e.g. at pH values above 9–9.5, the reaction of formaldehyde with phenols leads to nucleus-hydroxymethylated phenols to which the formaldehyde naturally is fixed irreversibly, thus being prevented from producing antimicrobial effects (see Figure 7). Additionally the introduction of the hydroxymethyl groups into the phenyl ring has an adverse effect on the antimicrobial activity of phenolic compounds.

In general the antimicrobial efficacy of the hemiformals is in conformity with their formaldehyde contents (exception: benzylalcoholhemiformal; 3.1.2).

This is not true for the formal which do not exhibit remarkable antimicrobial activity. Exception: (3-iodopropargyl)-(4-chlorophenyl) formal (3.1.6.) the antimicrobial activity of which is not based on the formaldehyde incorporated into the molecule. It can pass for a rule that formaldehyde releasing compounds are effective if their formaldehyde contents can be analysed in diluted aqueous solutions using the method described by (Tanenbaum & Bricker (1951) (reaction of the compounds with phenylhydrazine and potassium hexacyanoferrate). However,

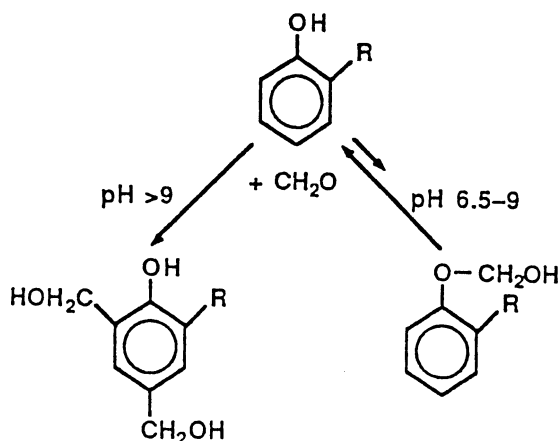


Figure 7 Reaction of phenol derivative with formaldehyde.

in case the formaldehyde content of a compound is not detectable by the Tanenbaum method, it may be effective in spite of that. Examples of such compounds are certain N-hydroxymethyl amides (3.4.).

The importance of hemiformals as preservatives for practical applications is based on their differing and superior chemico-physical properties as compared with formalin.

Microbicide group (substance class)	3.1. O-HYDROXYMETHYL COMPOUNDS
Chemical name	3.1.1. 1-Butanolhemiformal
Chemical formula	$C_5H_{12}O_2$
Structural formula	$CH_3-(CH_2)_2-CH_2-O-(CH_2-O)_xH$
Molecular mass	104.15 ($x = 1$; HCHO content 28.8%)
CAS-No.	3085-35-6
Synonym/common name	n-butylalcoholhemiformal, propylcarbinolhemiformal butyl-hydroxymethyl ether
Supplier	KOEI CHEMICAL
Chemical and physical properties	
Appearance	colourless fluid with the odour of formaldehyde
Content (%)	~90 ($x = 1.96$; HCHO content ~40)
Boiling point/range °C (101 kPa)	105
Density g/ml (20°C)	1.989
Flash point °C	58
Stability	decomposition by heating to the boiling point
Solubility g/l (20°C)	in water > 100; miscible with alcohols
Toxicity data	
See formaldehyde (2.1a.)	
Irritant to skin and mucous membranes.	

Antimicrobial activity/applications

The action of n-bunatolhemiformal (HCHO content 28.8%) corresponds to its formaldehyde content. Minimum inhibition concentrations:

300 mg/l for *Escherichia coli*

4.000 mg/l for formaldehyde resistant bacteria (Paulus, 1976).

n-Butanolhemiformal may be used as a preservative for water based products with the advantage that the affinity of formaldehyde to n-butanol is stronger than in formalin to water. Additionally n-butanol can serve as a formulation aid (e.g. solvent) with antimicrobial effectiveness, for example, when a microbicide has to be formulated to a preservative.

Microbicide group (substance class)	3.1. O-HYDROXYMETHYL COMPOUNDS
Chemical name	3.1.2. Benzylalcoholmono(poly)hemiformal (BHF)
Structural formula	$C_6H_5-CH_2-O-(CH_2O)_xH$ $x = 1.5$
Molecular mass	153
CAS-No.	14548-60-8
EC-No.	238-588-8; EEC-no. 55
Synonym/common name	(phenylmethoxy)-methanol, phenylcarbinol hemiformal, phenylmethanolhemiformal, benzylhemiformal
Supplier	BAYER AG, BODE CHEMIE, SCHÜLKE & MAYR
Chemical and physical properties	
Appearance	colourless liquid with a characteristic smell and a slight odour of formaldehyde (2.1.)
Content (%)	> 99 (HCHO content 29)
Boiling point/range °C (101 kPa)	105 (decomposition)

Solidification point °C	< -60
Density g/ml (20°C)	1.11
Vapour pressure hPa (20°C)	~10
Viscosity mPas (20°C)	14.7
Flash point °C	80
Auto ignition temperature °C	400
pH value (1% in water)	~5
Stability	releases formaldehyde when heated and in aqueous solution; stable at pH 3–12 (20°C)
Solubility g/l (20°C)	25 in water; soluble in organic solvents
Toxicity data (source: Bayer AG)	
LD ₅₀ oral	1.700 mg/kg rat (male)
dermal (exposure 4 h)	> 2.000 mg/kg rat (male)
dermal (exposure 4 h)	1.000–2.000 mg/kg rat (female)
LD ₅₀ inhalation (exposure 4 h)	> 0.5 mg/l for rats

Concentrated BHF causes irritation of the skin and eyes of rabbits (exposure 8 h).

Concentration of application (0.2%) is not irritant to the skin, but irritant to the eyes of rabbits. Sensitization may occur.

Ecotoxicity:

BHF is easily degradable (> 99% according to DOC-determination). Activated sludge organisms in sewage plants are not disturbed in their activity by BHF concentrations up to 500 mg/l in a respiric degradation test (ASCOMAT).

TOC value:	775
COD value	2.030
BOD ₅ value:	1.210

LC₀ for *leuciscus idus* 20 mg/l (exposure 48 h)

Antimicrobial effectiveness/applications

According to the MIC in Table 17 BHF has a higher degree of efficiency than one would conclude from its formaldehyde content. A careful examination of the spectrum of effectiveness reveals the phenomenon of synergism which makes itself felt in the case of bacteria, fungi and yeasts (Paulus, 1976). Additionally BHF offers the advantage that its efficacy is independent of pH value and is developed fully even in the presence of ionic or non-ionic agents. On the other hand the disinfecting power of BHF is slow compared to phenolic active ingredients or to quaternary ammonium compounds (Table 18).

The MIC in Table 17 show that the antimicrobial effectiveness of BHF against bacteria is stronger than against fungi and yeasts. When looking for possibilities to overcome this deficiency which is characteristic for most of the formaldehyde releasing compounds it is self suggesting to use phenolic microbicides as combination partners, which are known to have an excellent fungicidal effect.

A remarkable synergism which especially extends to fungi, but also to yeasts, is noticed, when BHF is combined with microbicidal phenol derivatives (Paulus et al., 1970b). This synergism is so striking that even additions of 15–20% phenol derivatives to BHF are sufficient to broaden the spectrum of activity to the desired extent. However, phenols are known to be able to react with formaldehyde and irreversible nucleus hydroxymethylations may occur under specific conditions. In this way the formaldehyde releasing compounds are deprived of microbicidal formaldehyde. In addition, the phenol derivatives hydroxymethylated in the phenyl ring are less effective than the corresponding starting products. However, these reactions, which reduce the antimicrobial effect of the mixing partners, can be suppressed as long as pH levels between 6 and 9, and max. 9.5 are available (Paulus, 1980).

Thanks to its excellent properties – low toxicity, good skin compatibility, negligible environmental toxicity, colourlessness, neutral reaction, efficacy independent of pH and unaffected by detergents or emulsifying agents – BHF is successfully employed in a variety of industrial fluids of widely differing compositions. Examples: cosmetics, polymer emulsions, pigment slurries, thickening solutions, emulsion paints, adhesive dispersions, metal working fluids, glues, biopolymers, concrete additives, wax emulsions, polishes and other water-based formulations.

Table 17 Minimum inhibition concentrations (MIC) of BHF in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	150
<i>Bacillus mycoides</i>	100
<i>Bacillus punctatus</i>	50
<i>Bacillus subtilis</i>	50
<i>Bacterium vulgare</i>	50
<i>Desulfovibrio desulfuricans</i>	50
<i>Escherichia coli</i>	100
Formaldehyde resistant bacteria ^a	2000
<i>Pseudomonas aeruginosa</i>	150
<i>Pseudomonas fluorescens</i>	130
<i>Staphylococcus aureus</i>	70
<i>Alternaria alternata</i>	500
<i>Aspergillus flavus</i> Link	240
<i>Aspergillus niger</i>	900
<i>Aspergillus terreus</i>	1000
<i>Aureobasidium pullulans</i>	700
<i>Chaetomium globosum</i> Kunze	300
<i>Penicillium glaucum</i>	1500
<i>Rhizopus nigricans</i>	600
<i>Sclerophoma pityophila</i>	500
<i>Trichoderma viride</i>	2000
<i>Candida albicans</i>	1000
<i>Candida crusei</i>	500
<i>Torula utilis</i>	400

^a Paulus (1979). The isolate is considered to be a strain of *Pseudomonas putida*. However, it is atypical in not producing acid from xylose.

Table 18 Disinfecting concentrations (%) of BHF and mixtures of BHF with phenolic microbicides, determined by the suspension method after 10 min

Active ingredient	Test organism		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
BHF	1.50	1.00	1.50
BHF/2-phenyl-phenol (4/1)	0.15	0.15	0.25
BHF/3-methyl-4-chlorophenol (4/1)	0.25	0.15	0.25
BHF/2,2'-dihydroxy-5,5'-dichlorodiphenylmethane (4/1)	0.04	0.07	0.25

BHF is registered in the EC list of preservatives for cosmetics with a maximum authorized concentration of 0.15% (for rinse-off products only).

The Plastic Commission of the Federal German Health Office (BGA) has included BHF in recommendation XXXVI as preservative for auxiliaries used in the manufacture of paper and board for the food industry and in recommendation XIV as a preservative for plastic dispersions.

In disinfectants BHF is used in combination with other active ingredients, preferably with phenol derivatives (see Table 18) or quaternary ammonium compounds (18.1). In chemical WCs' BHF has both a disinfecting and a deodorizing effect.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

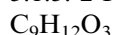
Synonym/common name

Chemical and physical properties

Appearance

3.1. O-HYDROXYMETHYL COMPOUNDS

3.1.3. 2-Phenoxyethanolhemiformal



168.17

41965-76-8

unknown

phenyl-hydroxymethyl-ethyleneglycolether

clear, colourless, slightly viscous fluid with a faint formaldehyde odour

Content (%)	> 99 (HCHO content 17.8)
Density g/ml (20°C)	1.113
Stability	releases formaldehyde when heated, and when diluted in water-based formulations
Solubility g/l (20°C)	in water approx. 30, miscible with alcohols and ketones
Toxicity data	
See paraformaldehyde (2.1a.) and 2-phenoxy-ethanol (1.7.).	

Antimicrobial effectiveness/applications

As is demonstrated in Table 19 2-phenoxy-ethanol hemiformal is much more effective than 2-phenoxy ethanol itself (1.7). It may be used alone or in combination with other active ingredients, especially those which exhibit a more fungicidal effect, for the in-can protection of cosmetic products, and also as a preservative for other industrial fluids. Although 2-phenoxy ethanol hemiformal is not mentioned in the EC positive list of preservatives for cosmetic products, there should be no inhibition for this application as it consists of 2-phenoxy ethanol and formaldehyde (addition product) which are registered in the a. m. EC list. Additionally, 2-phenoxy ethanol hemiformal may be regarded as a solvent with antimicrobial effectiveness which proves useful for the formulation of preservatives.

Table 19 Minimum inhibition concentrations (MIC) of 2-phenoxy ethanolhemiformal in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	500
<i>Pseudomonas aeruginosa</i>	1000
<i>Staphylococcus aureus</i>	500
<i>Candida albicans</i>	1000
<i>Aspergillus niger</i>	1500
<i>Chaetomium globosum</i>	500
<i>Penicillium glaucum</i>	2000

Microbicide group (substance class)	3.1. O-HYDROXYMETHYL COMPOUNDS
Chemical name	3.1.4. Ethyleneglycolhemiformals and ethyleneglycol-formal = 1,3-dioxolane

The addition of formaldehyde to ethyleneglycol=EG (1.13.) leads to EG-hemiformal (3.1.4a.) and EG-bishemiformal (3.1.4b.). Under acidic conditions water is eliminated from the EG-hemiformal and the formal 1,3-dioxolane (3.1.4c.) is formed (see Figure 8).

Chemical name	3.1.4a. EG-hemiformal
Chemical formula	C ₃ H ₈ O ₃
Structural formula	HO-CH ₂ -CH ₂ -O-CH ₂ -OH
Molecular mass	92.06
CAS-No.	13149-79-61
EC-No.	236-090-5

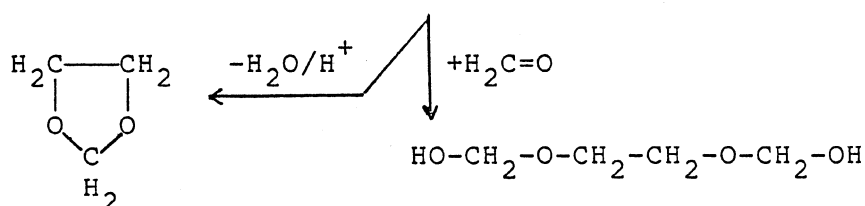
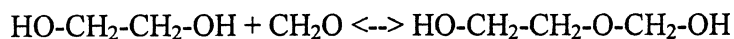
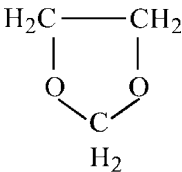


Figure 8 The reaction of formaldehyde with ethylene glycol (EG).

Synonym/common name	(2-hydroxyethyl)-hydroxymethyl-ether, 1-hydroxy-2-hydroxymethoxy-ethane, hydroxymethylmonoglycol-ether
Content (%)	> 99 (HCHO content 32.6)
Density g/ml (20°C)	1.17
Chemical name	3.1.4b. EG-bishemiformal
Chemical formula	C ₄ H ₁₀ O ₄
Structural formula	HO-CH ₂ -O-CH ₂ -CH ₂ -O-CH ₂ -OH
Molecular mass	122.08
CAS-No.	3586-55-8
EC-No.	222-720-6
Synonym/common name	glycol-bis(hydroxymethyl)-ether, ethylenedioxy-methanol, 1,6-dihydroxy-2,5-dioxahexane
Supplier	BODE CHEMIE, AB SVENSKA SHELL, TROY
Content	> 99 (HCHO content 42.2)
Density g/ml (20°C)	1.2
Vapour pressure hPa (20°C)	< 10
Refractive index nD (20°C)	1.436
Flash point °C	83
pH (concentrate at 20°C)	approx. 5
Further data to the properties of 3.1.4a. and 3.1.4b.	
Appearance	clear, colourless fluids with the odour of formaldehyde; formaldehyde is released, when heated and in aqueous solutions; the HCHO content of the compounds is completely detectable with the Tanenbaum method
Solubility	complete in water, alcohols and ketones
Toxicity data of 3.1.4a. and 3.1.4b.	
See 1,2-ethanediol (1.13.) and formaldehyde (2.1a.).	
LD ₅₀ oral for 3.1.4b.	761 mg/kg rat
Irritant to skin and mucous membranes.	

Antimicrobial effectiveness/applications

The fact that the antimicrobial activity of the EG-hemiformals (see Table 20) is in conformity with their formaldehyde contents, confirms that they in aqueous solutions easily disintegrate to the starting products formaldehyde and EG. Accordingly they are especially effective against bacteria and therefore used for the in-can protection of a large variety of industrial fluids, mainly together with other active ingredients, e.g. fungicides. EG-hemiformals are also of importance as formulation aids in preservatives.

Chemical name	3.1.4c. 1,3-Dioxolane
Chemical formula	C ₃ H ₆ O ₂
Structural formula	
Molecular mass	74.08
CAS-No.	646-06-0
EC-No.	211-463-5
EPA-TSCATS	Data Base Jan. 2001.
Synonym/common name	1,3-dioxo-cyclopentane, ethyleneglycolformal, ethyleneglycol-methyleneether
Supplier	SIGMA ALDRICH
Chemical and physical properties	
Appearance	clear, colourless fluid with aromatic odour
Content (%)	99 (HCHO content 40.5)

Boiling point/range °C (101 kPa)	75–76
Solidification point °C	–95
Density g/ml (20°C)	1.064
Vapour pressure hPa (20°C)	93.1
Refractive index nD (20°C)	1.401
Flash point °C	–3
Auto ignition temperature °C	274
Stability	does not release formaldehyde under mild conditions, however, is sensitive to acids
Solubility	complete in water, alcohols and ketones
Toxicity data	
LD ₅₀ oral	3.200 mg/kg mouse 5.200 mg/kg rabbit
LD ₅₀ inhalative (4 h)	20.650 mg/m ³ for rats 10.500 mg/m ³ for mice
LD ₅₀ dermal	15.000 mg/kg rat 8.480 ml/kg rabbit
Irritant to skin and mucous membranes.	

Antimicrobial effectiveness/application

The considerable formaldehyde content of 1,3-dioxolane is not detectable with the Tanenbaum method. Accordingly 1,3-dioxolane does not exhibit substantial antimicrobial effectiveness (see Table 20).

Table 20 Minimum inhibition concentrations (MIC) of EG hemiformals and EG formal

Test organism	MIC (mg/litre)		
	EG monohemiformal	EG bishemiformal	EG formal
<i>Escherichia coli</i>	500	350	> 5000
<i>Pseudomonas aeruginosa</i>	1500	1000	> 5000
<i>Staphylococcus aureus</i>	500	200	> 5000
<i>Candida albicans</i>	500	200	> 5000
<i>Aspergillus niger</i>	1000	500	> 5000
<i>Chaetomium globosum</i>	500	200	> 5000
<i>Penicillium glaucum</i>	2000	1000	> 5000

Microbicide group (substance class)	3.1 O-HYDROXYMETHYL COMPOUNDS
Chemical name	3.1.5. (2-(2-butoxyethoxy)ethoxy)methanol
Chemical formula	C ₉ H ₂₀ O ₄
Structural formula	C ₄ H ₉ -O-(CH ₂) ₂ -O-(CH ₂) ₂ -O-CH ₂ -OH
Molecular mass	192.25
CAS-No.	56289-76-0
EC-No.	260-097-2
Synonym/common name	diethyleneglycol monobutylether hemiformal
Supplier	SCHUELKE & MAYR

Chemical and physical properties

Appearance	clear, colourless fluid with the odour of formaldehyde
Content (%)	approx. 100 (HCHO content approx. 15)
Stability	decomposes easily by heating and in water based formulations to the starting products butyl diglycol (1.16) and formaldehyde (2.1.)
Solubility	highly soluble in water and organic solvents
Toxicity data	
See 2.1a. and 1.16.	

Antimicrobial effectiveness/applications

As a hemiformal the compound exhibits an antimicrobial activity which is in line with its formaldehyde content. In practice the hemiformal has mainly gained importance as a solvent disposing of antimicrobial efficacy.

Microbicide group (substance class)

Chemical name

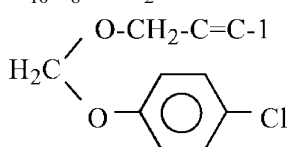
Chemical formula

Structural formula

3.1 O-HYDROXYMETHYL COMPOUNDS

3.1.6. (3-Iodopropargyl)-(4-chlorophenyl) formal

$C_{10}H_8ClIO_2$



Molecular mass

322.54

CAS-No.

29772-02-9

EC-No.

Unknown

Supplier

NAGASE CHEMICALS LTD

Chemical and physical properties

Appearance

pale brown fluid with an unpleasant odour

Content (%)

approx. 99 (HCHO content 9.3)

Boiling point/range °C (0.027 kPa)

130

Density g/ml (25°C)

1.572

Vapour pressure hPa (25°C)

< 1000

Viscosity mPa · s (25°C)

16

Refractive index nD (25°C)

1.583

Flash point °C

164

Stability

sensitive to acids: hydrolyzes to the starting products 3-iodopropargyl-alcohol (1.11.), 4-chlorophenol (7.5.1.) and formaldehyde (2.1.)

Solubility

sparingly soluble in water, easy soluble in organic solvents

Toxicity data

LD₅₀ oral

1.250 mg/kg rat

dermal

> 2.000 mg/kg rat

Irritant to skin and mucosa; skin irritation was not observed in tests with formulations containing less than 10% of the active ingredient (patch test, exposure: 24 h).

Fish toxicity: LC₀ for Killi fish

1.5 mg/l

Antimicrobial effectiveness/applications

(3-Iodopropargyl)-(4-chlorophenyl)formal is a powerful fungicide with a broad spectrum of activity but only a few species of bacteria are covered by the efficacy of the compound (see Table 21). In view of the MIC it is apparent that formaldehyde (being mainly effective against bacteria) does not take a significant part in the activity of the formal. More relation can be found to 3-iodopropargyl alcohol (1.11.) and 4-chlorophenol (7.5.1.) which can generate from the compound.

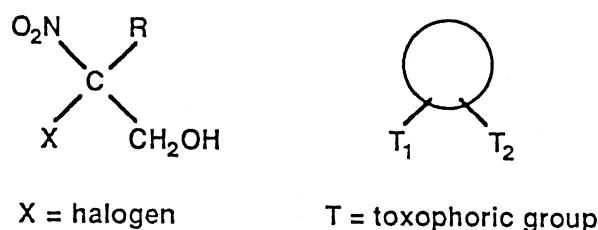
Formulations of the active ingredient are recommended for the protection of wood, leather, textiles, paints, paper. Because of the gaps in the activity spectrum it is not suitable to prevent bacterial deterioration in aqueous functional fluids. The compound also exhibits slimicidal and algicidal effectiveness.

3.2 C-Hydroxymethyl compounds

Activated hydrogen atoms, prepared to react with formaldehyde to yield C-hydroxymethyl compounds, one finds in nitro-hydrocarbons at the carbon atom adjacent to the nitro group, in ketones at the carbon atom in alpha-position towards the carbonyl group, in certain phenol derivatives. However, the formaldehyde combined in these substances cannot be determined by the Tanenbaum method, as the hydroxymethyl group is fixed to the substances more or less irreversibly. Accordingly C-hydroxymethyl compounds in general do not exhibit antimicrobial activity with the exception of the hydroxymethylated nitro-hydrocarbons, which under certain conditions (pH > 7) release formaldehyde, though very slowly. As C-hydroxymethylated nitro-hydrocarbons

Table 21 Minimum inhibition concentrations (MIC) of (3-Iodopropargyl)-(4-chlorophenyl)formal in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	25
<i>Aureobasidium pullulans</i>	25
<i>Aspergillus niger</i>	5
<i>Chaetomium globosum</i>	15
<i>Coniophora puteana</i>	0.5
<i>Lentinus tigrinus</i>	5
<i>Penicillium glaucum</i>	3.5
<i>Cladosporium cladosporioides</i>	1.5
<i>Sclerophoma pityophila</i>	7.5
<i>Trichoderma viride</i>	50
<i>Escherichia coli</i>	> 1000
<i>Pseudomonas aeruginosa</i>	> 1000
<i>Staphylococcus aureus</i>	20

**Figure 9** Molecule with two toxophoric groups.

are able to react with further formaldehyde, amines and amides, microbicides having a wide range of different chemical and physical properties can be synthesized within this class of substances. The antimicrobial activity of C-hydroxymethylated nitro-hydrocarbons can be considerably increased by introduction of an activated halogen atom in alpha-position to the electronegative, nitro group (see Figure 9). Such molecules possess two reactive sites (toxophoric groups) which can react with nucleophilic centers of the microbial cell.

The hydroxymethylated nitro-hydrocarbons disposing of an activated halogen are described under “17. Compounds with activated halogen atoms”.

Microbicide group (substance class)	3.2 C-HYDROXYMETHYL COMPOUNDS
Chemical name	3.2.1. Tris(hydroxymethyl)nitromethane
Chemical formula	$C_4H_9O_5$
Structural formula	$O_2N-C(CH_2OH)_3$
Molecular mass	151.12
CAS-No.	126-11-4
EC-No.	204-769-5
EPA-FIFRA	approval for antimicrobial applications
EPA-TSCATS	data base Jan. 2001
Synonym/common name	2-hydroxymethyl-2-nitro-1,3-propandiol, trimethylolnitromethane, Tris Nitro
Supplier	DOW-ANGUS, SIGMA ALDRICH
Chemical and physical properties	
Appearance	white, crystalline and practically odourless
Content (%)	~99 (HCHO content 60)
Melting point °C	175–176
pH (0.1M in water at 20°C)	5.0
Stability	very stable when dry, and in media with pH values up to 6.5; releases formaldehyde increasingly with pH and temperature; at 20°C in solutions buffered to pH 8 9%, and in those buffered to pH 9 12% of the formaldehyde content are detectable by the Tanenbaum method (Paulus, 1980)

Solubility g/l (20°C) 2.200 in water; very soluble in alcohols, practically insoluble in hydrocarbons such as heptane, kerosene, mineral oil, benzene, toluene

Toxicity data

Acute toxicity for a 50% aqueous solution (Source: DOW-ANGUS)

LD₅₀ oral 1.875 mg/kg rat
 LD₅₀ dermal > 2.000 mg/kg rabbit
 LD₅₀ inhalative (4 h) 2.4 mg/l for rats

In tests with rabbits non irritant to the skin and mucous membrane.

Minimal sensitization potential.

Not mutagenic in three different test methods.

Ecotoxicity:

Not readily biodegradable (closed bottle test).

Inhibition of activated sludge organisms at a concentration of 96 mg/kg sludge/120 h (Sapromat test).

LC₅₀ for rainbow trout 410 mg/l/96 h
 bluegill sun fish > 300 mg/l/96 h
 daphnia magna 50 mg/l/24 h

EC₅₀ for algae (scenedesmus) 64.3 mg/l/72 h

EC₀ for bacteria (*Pseudomonas aeruginosa*) 300 mg/l/16 h

COD value 1.120 mg/l

BOD5 value 8 mg/l

Antimicrobial effectiveness/applications

Mould producing fungi are more tolerant to Tris Nitro than bacteria. However, Tris Nitro and other hydroxymethyl-hydrocarbons cannot be ranked among the very effective formaldehyde releasing compounds, although in alkaline media there is a noticeable increase of the tendency, especially of Tris Nitro, to release formaldehyde, accompanied by a corresponding increase in efficacy. A report of Clark et al. (1984) surveys the synthesis and antimicrobial activity of aliphatic nitro compounds. The MIC in Table 22 which were determined at pH 7.4 are significantly higher than one would expect regarding the formaldehyde content of Tris Nitro.

Tris Nitro as a preservative for a great variety of industrial fluids has been widely substituted by more effective formaldehyde releasing compounds. However, its good skin compatibility and the fact that there is at no time any detectable odour of formaldehyde is not a negligible advantage, especially when the compound is applied for the protection of metal working fluids.

Table 22 Minimum inhibition concentration (MIC) of a 50% aqueous solution of tris nitro at pH 7.4 (Source: DOW-ANGUS)

Test organism	MIC (mg/l)
<i>Bacillus subtilis</i>	250–500
<i>Desulfovibrio desulfuricans</i>	< 33
<i>Enterobacter aerogenes</i>	65–125
<i>Escherichia coli</i>	500–1000
<i>Micrococcus luteus</i>	125–250
<i>Pseudomonas aeruginosa</i>	500–1000
<i>Salmonella typhi</i>	65–125
<i>Staphylococcus aureus</i>	65–125
<i>Streptococcus faecalis</i>	250–500
<i>Aspergillus niger</i>	> 2000
<i>Aureobasidium pullulans</i>	> 2000
<i>Candida albicans</i>	> 2000
<i>Cephalosporium sp</i>	1000–2000
<i>Cladosporium herbarum</i>	> 2000
<i>Fusarium moniliforme</i>	> 2000
<i>Saccharomyces cerevisiae</i>	500–1000
<i>Trichophyton mentagrophytes</i>	250–500

Microbicide group (substance class)	3.2 C-HYDROXYMETHYL COMPOUNDS
Chemical name	3.2.2. Bis(hydroxymethyl)-trifluoromethyl-nitromethane (Bassner et al., 1988)
Chemical formula	$C_4H_6F_3NO_4$
Structural formula	$(HOCH_2)_2CNO_2-CF_3$
Molecular mass	189.10
CAS-No.	3857-06-5
EC-No.	—
Synonym/common name	2-nitro-2-trifluoromethyl-1,3-propanediol
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	> 99 (HCHO content 32)
Melting point °C	128–130
Solubility	Sparingly soluble in water, soluble in acetone, DMF
Toxicity data	
Not yet available.	

Antimicrobial effectiveness/applications

The MIC's listed in Table 23 demonstrate, that the antimicrobial activity of this bis-hydroxymethylated nitromethane derivative does not correspond to its formaldehyde content. The spectrum of effectiveness covers bacteria and fungi and is interesting in its equality; it is in that respect superior to that of Bronopol (17.14.).

2-Trifluoromethyl-2-nitro-propane-1,3-diol may be used as a broad spectrum preservative for industrial fluids, e.g. detergents, cosmetics, polymer emulsions and other water based formulations which can be deteriorated due to microbial activity.

Table 23 Minimum inhibition concentrations (MIC) of 2-Trifluoromethyl-2-nitro-propane-1,3-diol in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	500
<i>Aeromonas punctata</i>	500
<i>Bacillus mycoides</i>	500
<i>Bacillus subtilis</i>	500
<i>Escherichia coli</i>	200
<i>Leuconostoc mesenteroides</i>	500
<i>Proteus mirabilis</i>	200
<i>Pseudomonas aeruginosa</i>	350
<i>Pseudomonas fluorescens</i>	500
<i>Staphylococcus aureus</i>	200
<i>Alternaria alternate</i>	200
<i>Aspergillus niger</i>	500
<i>Aureobasidium pullulans</i>	500
<i>Chaetomium globosum</i>	350
<i>Cladosporium cladosporioides</i>	200
<i>Penicillium glaucum</i>	500
<i>Sclerophoma pityophila</i>	200
<i>Trichoderma viride</i>	500

Microbicide group (substance class)	3.2 C-HYDROXYMETHYL COMPOUNDS
Chemical name	3.2.3. Mixture of 70% N-(2-nitrobutyl)morpholine (A) and 20% N,N'-(2-ethyl-2-nitrotrimethylene)dimorpholine (B)
Chemical formula	A: $C_8H_{16}N_2O_3$ B: $C_{13}H_{25}N_3O_4$
Structural formula	

Molecular mass	A: 188.23	B: 287.36
CAS-No.	A: 2224-44-4	B: 1854-23-5
EC-No.	A: 218-748-3	B: 217-450-0
EPA-Reg.	for use as an antimicrobial agent in industrial functional fluids, e.g. in metal working fluids	
Synonym/common name	A: 4-(2-nitrobutyl)morpholine	B: 4,4'-(2-ethyl-2-nitropropane-1,3,-diyl)bismorpholine
Supplier	DOW-ANGUS	

The morpholine derivatives A and B are listed under "C-hydroxymethyl compounds" because monomethylol nitropropane and dimethylol nitropropane are the intermediates in synthesis, which react with morpholine to form the end products.

Appearance	yellowish-brown liquid with a faint amine odour
Content%	approx. 90; HCHO content: A: 15.9 B: 20.9
Boiling point/range °C (101 kPa)	> 200
Solidification point °C	approx. 10
Density g/ml (25°C)	1.076
Vapour pressure hPa (90°C)	39.9
Refractive index nD (20°C)	1.472
Viscosity mPas (20°C)	40
Flash point °C	87
pH value (20°C)	9.5–10.0
Partition coefficient (log Pow)	0.92
Stability	sensitive to acids; decomposition starts at pH < 6.5; stable and effective at a pH of 7.0 or above
Solubility g/l (20°C)	11 in water; soluble in organic solvents including non polar solvents

Toxicity data (Source: DOW-ANGUS)

LD ₅₀ oral	625 mg/kg rat
LD ₅₀ dermal	420 mg/kg rabbit

The concentrated product is irritant to the skin and corrosive to mucous membranes. 0.1% in a metal working fluid caused no irritation (24 hours patch test with humans). – Sensitization may occur.

The product is regarded as non-mutagenic.

Results of subacute-chronic toxicity tests with rats:

90-day-dermal toxicity: no systemic effect at 1000 mg/kg/day

Teratogenicity study, oral application: no teratogenic effect at 100 mg/kg/day

Exposure limits (occupational): see formaldehyde (2.1.).

Ecotoxicity:

LC ₅₀ for	rainbow trout	1.1 mg/l (96 h)
	bluegill sun fish	1.3 mg/l (96 h)
	daphnia magna	1.9 mg/l (48 h)
EC ₁₀ for bacteria (<i>Pseudomonas putida</i>)		37 mg/l (16 h)
COD-value:	1720 mg/g	
BOD5-value:	28 mg/g	

Antimicrobial effectiveness/application

The broadness of the spectrum of effectiveness is noteworthy. The a.m. mixture of morpholine derivatives is distinguished by high solubility in non-polar solvents, an advantage which is of value, when the mixture has to be incorporated into oil concentrates (e.g. lubricoolants) as an antimicrobial ingredient that is effective as a preservative in the emulsions prepared later out of such oil concentrates in water. It is recommended to establish concentrations between 500 and 1000 ppm of a.i. in such emulsions for a good protection against microbial deterioration.

Table 24 Minimum inhibition concentrations (MIC) of the mixture of morpholine derivatives (Source: DOW-ANGUS)

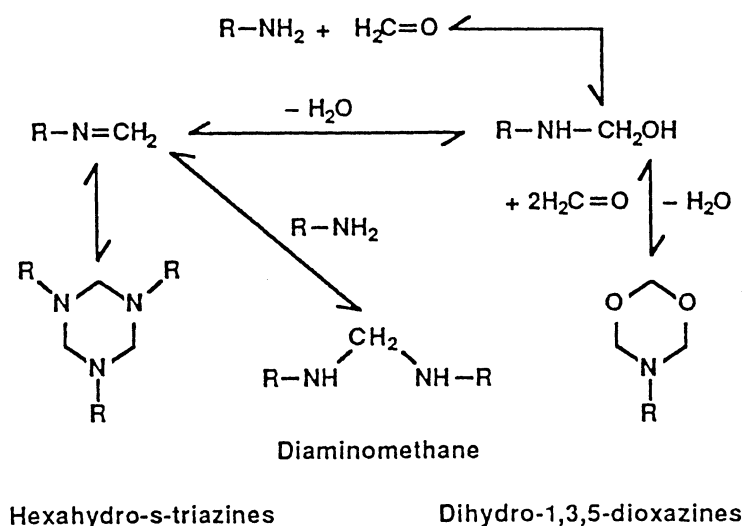
Test organism	MIC (mg/l)
Bacteria	
<i>Staphylococcus aureus</i>	100–500
<i>Streptococcus faecalis</i>	100–500
<i>Streptococcus hemolyticus</i>	100–500
<i>Escherichia coli</i>	100–500
<i>Pasteurella pseudotuberculosis</i>	10–100
<i>Pseudomonas aeruginosa</i>	100–500
<i>Desulfovibrio desulfuricans</i>	50–100
<i>Bacillus subtilis</i>	500–1000
Fungi	
<i>Candida albicans</i>	16–32
<i>Penicillium levitum</i>	125–250
<i>Aspergillus niger</i>	125–250
<i>Fusarium sp.</i>	500–1000
<i>Aureobasidium pullulans</i>	2000
<i>Cephalosporium sp.</i>	1000–2000
Algae	
<i>Plectonema boryanum</i>	2–4
<i>Oscillatoria prolifera</i>	4–9
<i>Chlorella pyrenoidosa</i>	1
<i>Anabaena flos-aquae</i>	< 16
<i>Selenastrum capricornutum</i>	< 16
<i>Nitzschia closterium</i>	< 16

3.3 Amine-formaldehyde-reaction-products

Products obtained by reaction of formaldehyde and amines are always characterized by, among other properties, antimicrobial effectiveness. Here, too, the introduction of the methylol group is the first reaction step; it may be followed by water being split off intra- or intermolecularly or by reaction with other formaldehyde molecules, thus allowing a wide variety of formaldehyde releasing compounds to be obtained (see Figure 10).

Probably the first synthesized formaldehyde releasing compound is hexamethylenetetramine (HTA) which was obtained in 1860 from the condensation of ammonia and formaldehyde according to the reaction pathway illustrated in Figure 11.

The antimicrobial efficacy of the amine – formaldehyde reaction products essentially corresponds to the formaldehyde content of these compounds. Known exceptions are HTA, hexahydro-oxadiazines and octahydro-s-tetrazines, which derive from ammonia respectively certain alkylolhydrazines (Paulus, 1980); here the detection of formaldehyde by the Tanenbaum methods gives a negative result, which means that these substances have no significant antimicrobial effect at neutral to alkaline pH; they release formaldehyde in acidic media only. This pH dependency is broken off, if, for example, HTA is quaternized (Jacobs et al., 1916). In contrast to HTA the quaternary hexaminium salts release formaldehyde widely independent of pH and therefore may be used as

**Figure 10** Reaction of formaldehyde with amines (Paulus, 1980).

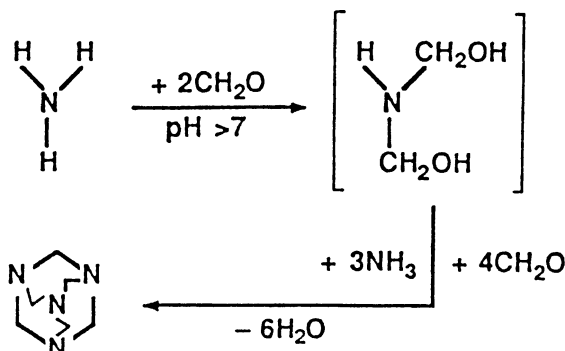
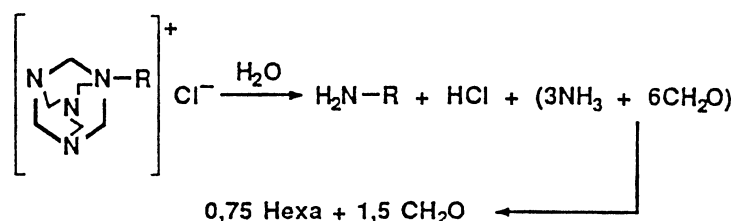
Figure 11 Condensation of NH_3 and CH_2O to hexamethylenetetramine.

Figure 12 Hydrolytic cleavage of quaternary hexaminium salts.

Table 25 MIC of quaternary hexaminium salts for *Bacillus subtilis* found and calculated from the CH_2O content of the compounds

Compound	MIC (mg/litre)		
	Found MIC	Calculated MIC	MIC corresp. to 25% calcul. efficacy
$[\text{Hexa-CH}_2\text{-CH=CHCl}]^+ \text{Cl}^-$	100	21	84
$[\text{Hexa-CH}_2\text{-CO-NH}_2]^+ \text{Cl}^-$	100	20	80
$[\text{Hexa-CH}_2\text{-CO-NH-CH}_2\text{OH}]^+ \text{Cl}^-$	50	18	72
Formaldehyde	15	—	—

preservatives also for media of neutral to alkaline pH. They are not comparable with the surface active conventional quaternary ammonium compounds (18.1.) as their antimicrobial effectiveness is based on the release of formaldehyde. The efficacy is however lower than one would expect considering the formaldehyde content of the hexaminium salts (Scott & Wolf, 1962). So it has been observed that in aqueous solutions, especially with pH values above 7, the hexaminium salts' antimicrobial efficacy rapidly decreases to quickly stabilize at a constant inhibition value. This phenomenon is explained by Figure 12 which illustrates how the hydrolytic cleavage takes place.

The 3 M NH_3 and 6 M CH_2O first liberated during hydrolytic cleavage at pH values >7 will soon become rearranged to form 0.75 M hexamethylenetetramine and 1.5 M CH_2O , the latter remaining available for the antimicrobial efficacy. Examination of MICs of different quaternary hexaminium salts confirms that in actual fact a mere 25% (approx.) of the hexaminium salts' calculated formaldehyde content is available for the antimicrobial efficacy (see Table 24).

Primary 2-hydroxy-alkylamines react with formaldehyde, as is demonstrated in Figure 13, at first to the corresponding N-hydroxymethyl compound which under the separation of water forms the N-methylene compound as an intermediate; the latter cyclizes to 1,3-oxazolidine, or trimerizes exothermically to 1,3,5-hexahydrotriazine. As the 1,3-oxazolidine thus formed disposes of a N-H group, it can react with additional formaldehyde to the corresponding dioxazolidinylmethane.

Secondary 2-hydroxy-alkylamines react with formaldehyde to 1,3-oxazolidines, too, but constitutionally not to dioxazolidinylmethanes, or 1,3,5-hexahydro-triazines.

The 1,3-oxazolidines listed here are highly soluble in water and polar solvents, and in oils, too. Aqueous solutions have a basic pH. The formaldehyde releasable is detectable by the Tanenbaum method; in consequence the antimicrobial effectiveness of the 1,3-oxazolidines corresponds to their formaldehyde content.

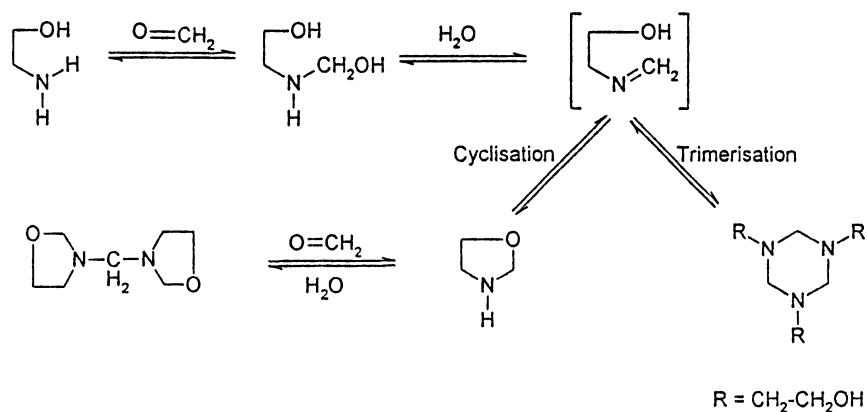


Figure 13 Reaction scheme for the formation of 1,3-oxazolidines and dioxazolidinyl methanes.

Formaldehyde releasing compounds of superior, and above all, more balanced antimicrobial effectiveness are obtained by reacting hydroxy-group containing formaldehyde releasing compounds and arylisocyanates, provided that the hydroxyl group does not form part of a hydroxymethyl group that may be split off. The microbicides thus formed are formaldehyde releasing N-aryl-carbamates (Paulus et al., 1975).

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCA

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content%

Melting point °C

Vapour pressure hPa (20°C)

Flash point °C

PH (0.2 M in H₂O)

Stability

Solubility g/l (20°C)

Toxicity data

LD₅₀ oral

Subcutaneous

LD₅₀ subcutaneous

LD₅₀ intravenous

Irritant to skin and mucosa.

No carcinogenic effects when applied orally.

ADI value: 0–0.15 mg/kg/day.

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

3.3.1. Hexamethylenetetramine (HTA)

C₆H₁₂N₄



140.19

100-57-0

202-905-8; EEC-no. 30

Test Submission Data Base Jan. 2001

1,3,5,7-tetraazaadamantane, Hexamine, Methenamine, Urotropin

MERCK INC., ISP, SIGMA-ALDRICH

white, crystalline, hygroscopic, odourless powder

99.5 (HCHO content 6M)

280 (sublimates at 230–270°C without melting)

< 0.013

250

8.4

stable at neutral and basic pH; releases formaldehyde in acidic media

680 in H₂O, 28 in ethanol; very soluble in chloroform, insoluble in ether

569 mg/kg mouse

215 mg/kg mouse

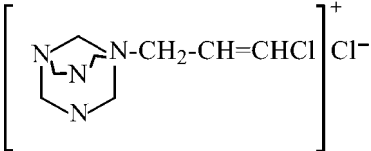
200 mg/kg rat

9.200 mg/kg rat

Antimicrobial effectiveness/applications

The efficacy at HTA is based on its formaldehyde content which, however, is only released in acid media. According to its composition HTA is the formaldehyde releasing compound with the highest percentage of bound formaldehyde. As with formaldehyde, HTA preferably attacks bacteria and not mould producing fungi to such an extent.

HTA is listed in the EC list of preservatives for cosmetics (maximum authorized concentration 0.15%). Percentage of use in US cosmetic formulations: 0.005%. The application is very limited, as HTA is only effective in acid formulations. As a preservative for acid food preparations or technical functional fluids, HTA today is no longer of importance.

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.2. 1-(3-Chloroallyl)-3,5,7-triaza-1-azoniaadamantane-chloride
Chemical formula	$C_9H_{16}Cl_2N_4$
Structural formula	
Molecular mass	251.16
CAS-No.	51229-78-8 (> 97%)/4080-31-3 (67%)
EC-No.	223-805-0 (67%); EEC-no. 31
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	N-(3-chloroallyl)-hexaminium-chloride, Quaternium-15
Supplier	DOW
Chemical and physical properties	
Appearance	White to off-white powder with a slight amine odour
Content (%)	> 97 (HCHO content approx. 71)
Melting point °C	decomposition starts at 60°C
Vapour pressure hPa (20°C)	negligible
Auto ignition temperature °C	390
pH of fresh aqueous solutions	4.5 to 5.5 (drifts to an equilibrium of pH of 6.5 to 7.5)
Stability	sensitive to heat (> 40°C); releases formaldehyde in aqueous solutions widely independent of pH
Solubility g/l (25°C)	in: water 1272; methanol (anhydrous) 208; propylene glycol 187; glycerine (99,5%) 126; ethanol (absol.) 20.4; isopropanol (anhydrous) < 1; mineral oil < 1
Toxicity data (source: DOW CHEMICAL CO.)	
LD ₅₀ oral	1.550 mg/kg rat 78 mg/kg rat
LD ₅₀ dermal (powder)	2.877 mg/kg rabbit
(solution)	600 mg/kg rabbit
Irritant to skin and mucous membranes.	
Sensitization is possible at concentrations greater than 1% in H ₂ O.	
In vitro mutagenicity studies were negative.	
Ecotoxicity:	
LC ₅₀ for fish	26–48 mg/l
LC ₅₀ for Daphnia magna	1–10 mg/l

Not readily biodegradable according to OECD/EC guidelines. Biodegradation reached in Closed Bottle Test after 20 days: 38%.

Antimicrobial effectiveness/applications

The efficacy of N-(3-chloroallyl)-hexaminium chloride covers a broad spectrum of bacteria and mould producing fungi, yeasts included. However, the activity against bacteria is more pronounced than that against fungi, characterizing the microbicide as a formaldehyde releasing compound (see Table 26).

Table 26 Minimum inhibition concentrations (MIC) of N-(3-chloroallyl)-hexaminium chloride (67.5%)* in nutrient agar (Source: Dow Chemical Co.)

Test organism	ATCC	MIC (mg/l)
Bacteria		
<i>Bacillus subtilis</i>	8473	80
<i>Enterobacter aerogenes</i>	13048	80
<i>Escherichia coli</i>	11229	80
<i>Klebsiella pneumoniae</i>	8308	80
<i>Proteus vulgaris</i>	881	40
<i>Pseudomonas aeruginosa</i>	10145	240
<i>Pseudomonas aeruginosa PRD10</i>	15442	160
<i>Salmonella choleraesuis</i>	10708	80
<i>Staphylococcus aureus</i>	6538	40
Fungi and Yeast		
<i>Aspergillus niger</i>	16404	500
<i>Penicillium chrysogenum</i>	9480	500
<i>Pullularia pullulans</i>	16622	500
<i>Saccharomyces cerevisiae</i>	4105	250

*contains 32.5% Na₂HCO₃ as inert material

The hexaminium salt is not inactivated by cationic, anionic or nonionic formulation ingredients. The formaldehyde which is responsible for the antimicrobial efficacy is released independent of the pH of the substrate. Consequently N-(3-chloroallyl)-hexaminium chloride is a suitable preservative for many different water based formulations where it may be used independent of system pH between 4–10. In concentrations from 0.05 to 0.25% the preservative is applied for the in-can or in-tank protection of polymer emulsions, wet paper coatings, starches, inks, detergents, concrete additives and numerous other water based systems. According to its salt character and its high water solubility the N-(3-chloroallyl)-hexaminium salt does not tend to concentrate in the organic phase of oil and water emulsion systems but remains in the aqueous phase where the vegetation of microbes is likely to arise. The application should, however, be restricted to formulations which do not contain protein (e.g. casein) and to those which tolerate the tendency of the hexaminium salts to cause a little bit of yellowing especially in formulations containing traces of monomers which may react with the amine compounds released from hexaminium salts.

N-(3-chloroallyl)-hexaminium chloride is listed in the EC list of preservatives for cosmetics with a maximum addition rate of 0.2%. – Percentage of use in US cosmetic formulations: 3.52%.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

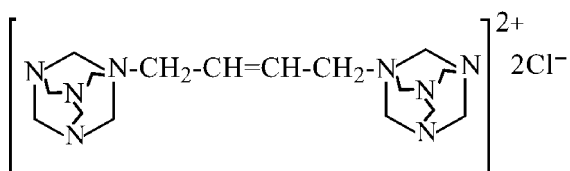
Chemical name

3.3.3. 1,1'-(2-Butenylene)-bis-(3,5,7-triaza-1-azoniaadamantane chloride)

Chemical formula

C₁₆H₃₀Cl₂H₈

Structural formula



Molecular mass

405.43

CAS-No.

51350-84-6

EC-No.

unknown

Supplier

COSAN CHEMICAL CORP.

Chemical and physical properties

Appearance

white, flowable, crystalline, odourless powder

Content (%)	96 (HCHO content 88.8)
Melting point °C	150 (decomposition)
pH (2% in water)	8.5–9
Stability	stable in the solid state, releases formaldehyde in aqueous solutions widely independent of pH
Solubility	highly soluble in water, moderately soluble in polar solvents, practically insoluble in non-polar solvents, e.g. oils
Toxicity data (Luloff & Eilender, 1975)	
LD ₅₀ oral	3.400 mg/kg rat
dermal	8.000 mg/kg rabbit

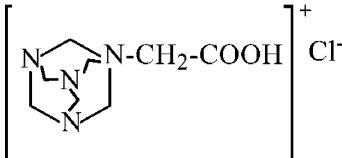
Antimicrobial effectiveness/applications

The antimicrobial efficacy corresponds with the formaldehyde which is released and disposable from the molecule (see 3.3.). Although there are two hexaminium moieties in the molecule of the a. i., compared with the N-(3-chloroallyl)-hexaminium salt one can expect an increase in efficacy of 20% only, due to the considerable ascent of the molecular weight.

The quaternary bis-hexaminiun salt described here is particularly useful as a preservative in a wide variety of water based systems, e.g. polymer emulsions, latex paints, pigment and dye slurries, concrete additives, starches, thickener solutions. Addition rates: 0.05–0.2%. For the rest what is mentioned before under 3.3. and 3.3.2. is valid.

Table 27 Minimum inhibition concentrations (MIC) of 1,1'-(2-Butylene)-bis-(3,5,7-triaza-1-azoniaadamantane chloride) in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	125
<i>Bacillus subtilis</i>	60
<i>Escherichia coli</i>	250
<i>Pseudomonas aeruginosa</i>	250
<i>Salmonella choleraesuis</i>	60
<i>Staphylococcus aureus</i>	125

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.4. 1-Carboxymethyl-3,5,7-triaza-1-azoniaadamante chloride
Chemical formula	C ₈ H ₁₅ ClN ₄ O ₂
Structural formula	
Molecular mass	234.69
CAS-No.	92623-86-4
EC-No.	unknown
Supplier	BUCKMANN

Chemical and physical properties of the 40% sodium salt solution

Appearance	clear, brown liquid
Content (%)	40 (HCHO content approx. 28)
Density g/ml (20°C)	1.19
pH (0.01% in water)	5.5–7.0
Stability	releases formaldehyde widely independent of the substrate
Solubility	highly soluble in water, soluble in polar solvents

Toxicity data for the 40% Na-salt solution (source: Buckman)

LD ₅₀ oral	> 5.000 mg/kg rat
dermal	> 2.000 mg/kg rabbit

Antimicrobial effectiveness/applications

The antimicrobial activity of the a. i. as demonstrated in Table 28 is exhibited over a broad pH range. The N-carboxymethyl-hexaminium salt is a zwitterionic substance that can be used as a preservative for aqueous functional fluids containing nonionic, anionic, or cationic components without deactivation or problems of incompatibility. Addition rates: 0.1–0.3%.

Table 28 Minimum inhibition concentrations (MIC) of the 40% aqueous sodium salt solution in nutrient agar

Test organism	MIC (mg/litre)
<i>Bacillus subtilis</i>	80
<i>Enterobacter aerogenes</i>	100
<i>Escherichia coli</i>	120
<i>Klebsiella pneumoniae</i>	80
<i>Proteus vulgaris</i>	100
<i>Pseudomonas aeruginosa</i>	200
<i>Salmonella choleraesuis</i>	80
<i>Staphylococcus aureus</i>	80

Microbicide group (substance class)

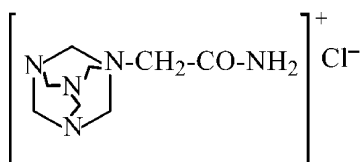
3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name

3.3.5. 1-Carbamoylmethyl-3,5,7-triaza-1-azoniaadamante chloride

Chemical formula

Structural formula



Molecular mass

233.70

CAS-No.

92988-93-7

EC-No.

unknown

Chemical and physical properties

Appearance

white, crystalline, odourless powder

Content (%)

approx. 100 (HCHO content 77)

Melting point °C

164 (decomposition)

Stability

stable in the solid state, releases formaldehyde in aqueous solutions independent of pH

Solubility

highly soluble in water, moderately soluble in polar solvents, practically insoluble in non-polar solvents, e.g. oils

Toxicity data

up to now unknown

Antimicrobial effectiveness/applications

The antimicrobial activity depends on the release of formaldehyde over a wide pH range as is already described under 3.3. – 3.3.4.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name

3.3.6 1-[(N-hydroxymethyl-carbamoyl)-methyl]-3,5,7-triaza-1-azoniaadamantane chloride

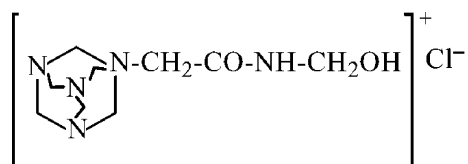
Chemical formula

C₉H₁₈ClN₅O₂

Table 29 Minimum inhibition concentrations (MIC) of *N*-carbamoylmethyl-hexaminium chloride in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	100
<i>Bacillus subtilis</i>	100
<i>Escherichia coli</i>	120
<i>Pseudomonas aeruginosa</i>	120
<i>Salmonella typhosa</i>	100
<i>Staphylococcus aureus</i>	100
<i>Aspergillus niger</i>	500
<i>Penicillium glaucum</i>	1000
<i>Rhizopus nigricans</i>	250

Structural formula



Molecular mass

263.72

CAS-No.

67508-69-4

EC-No.

unknown

Synonym/common name

N-(N'-hydroxymethyl)-carbamoylmethyl-hexaminium chloride

Chemical and physical properties

Appearance

white, crystalline, odourless powder

Content (%)

approx. 100 (HCHO content 79.6)

Melting point °C

155 (decomposition)

pH (5 g/l H₂O)

approx. 5

Stability

stable in the solid state; releases formaldehyde in aqueous solutions widely independent of pH

Solubility g/l (20°C)

in: methanol 47, ethylene glycol 19, acetone 0.08, cyclohexane 0.02; highly soluble in water

Toxicity data for a 70% aqueous solution (source: Bayer AG)

LD₅₀ oral

> 5.000 mg/kg rat

intravenous

> 1.000 mg/kg rat

dermal

> 1.000 mg/kg rat

No primary skin irritation; slightly irritant to mucous membranes.

Antimicrobial effectiveness/applications

N-(N'-hydroxymethyl)-carbamoylmethyl-hexaminium chloride is compared with other quaternary hexaminium salts of superior efficacy as the starting material for quaternizing hexamethylenetetramine in this case is a formaldehyde releasing compound, too, namely N-hydroxymethyl-chloracetamide (3.4.1.) – Applications as described under 3.3.2. – 3.3.4. – Addition rates: 0.05–0.2%.

3.3.7 Other quaternary hexaminium salts (Scott & Wolf, 1962).

	CAS-No.
N-methyl-hexaminium chloride	76902-90-4
N-benzyl-hexaminium chloride	5400-93-1
N-tolymethyl-hexaminium chloride	97159-48-3
N-(3,4-dichloro-) benzyl-hexaminium chloride	96433-36-2
N-(4-chloro-)benzyl-hexaminium chloride	96634-13-8
N-propyl-hexaminium bromide	92987-56-9
N-propylene-hexaminium chloride	35511-29-6
N-propargyl-hexaminium bromide	13496-17-8
N-butyl-hexaminium bromide	94584-23-3

Table 30 Minimum inhibition concentrations (MIC) of N-(N'-hydroxy methyl)-carbamoylmethyl-hexaminium chloride in nutrient agar

Test organism	MIC (mg/litre)
<i>Bacillus mycoides</i>	120
<i>Bacillus subtilis</i>	50
<i>Bacterium punctatum</i>	120
<i>Escherichia coli</i>	80
<i>Proteus vulgaris</i>	80
<i>Pseudomonas aeruginosa</i>	120
<i>Pseudomonas fluorescens</i>	120
<i>Staphylococcus aureus</i>	120
<i>Aspergillus flavus</i>	180
<i>Aspergillus niger</i>	400
<i>Aureobasidium pullulans</i>	250
<i>Chaetomium globosum</i> K.	180
<i>Penicillium glaucum</i>	800
<i>Rhizopus nigricans</i>	250

The list is not complete and may be continued.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name

3.3.8. 2-[(Hydroxymethyl)amino]alkanols

Structural formula

HO-CH₂-N- $\overset{\diagup}{\underset{\diagdown}{C}}$ -C-OH

2-[(Hydroxymethyl)amino]alkanols result from the addition of formaldehyde to 2-hydroxy-alkylamines. Such compounds are the intermediates in the formation of 1,3-oxazolidines (e.g. 3.3.10.) or hexahydro-s-triazines (e.g. 3.3.18.); see Figures 13 and 10.

Chemical name

3.3.8a. 2-[(Hydroxymethyl)amino]ethanol

Chemical formula

C₃H₉NO₂

Structural formula

HO-CH₂-NH-CH₂-CH₂-OH

Molecular mass

75.11

CAS-No.

34375-28-5

EC-No.

251-974-0

EPA-Reg.

approval for antimicrobial applications

Synonym/common name

2-hydroxyethyl-aminomethanol

Supplier

DEGUSSA-CREANOVA-COLORENTREND, TROY

Chemical and physical properties

Appearance

clear, colourless to light yellow fluid with a pungent odour

Content (%)

~100 (HCHO content ~40)

Boiling point/range °C (101 kPa)

113

Density g/ml (20°C)

1.135–1.165

Vapour pressure hPa (20°C)

9.9 (solvent)

Flash point °C

> 110

pH (10% i. H₂O) at 20°C

9.5–11.5

Stability

sensitive to acids; releases formaldehyde in aqueous systems

Solubility

complete in water, highly soluble in polar solvents

Toxicity data (source: DEGUSSA)

LD₅₀ oral

1620–1956 mg/kg rat

dermal

> 2000 mg/kg rat

LC₅₀ inhalative (aerosol)

0.62 mg/l (4 h) for rats

Caustic effect on skin, mucous membranes and eyes – No sensitizing effects are known.

Antimicrobial effectiveness/applications

The microbicide is recommended for use as an industrial preservative for the protection of water-based functional fluids with pH values > 7 such as latex paints, resin emulsions, adhesives, pigment slurries, concrete additives, metalworking fluids. Its compatibility with protein containing formulations is very limited. Normal use levels range between 0.1 and 0.3%. The spectrum of efficacy (see Table 31) covers above all bacteria as is characteristic for a formaldehyde releasing compound.

Table 31 Minimum inhibition concentrations (MIC) of 2-[(hydroxymethyl)amino]ethanol (Source: TROY)

Test organism	MIC (mg/l)
<i>Pseudomonas aeruginosa</i>	200–300
<i>Escherichia coli</i>	200–300
<i>Bacillus subtilis</i>	100–200
<i>Staphylococcus aureus</i>	100–200
<i>Enterobacter aerogenes</i>	100–200
<i>Proteus vulgaris</i>	50–100
<i>Streptococcus faecalis</i>	200–300
<i>Desulfovibrio desulfuricans</i>	10–30
<i>Candida albicans</i>	> 1000
<i>Saccharomyces cerevisiae</i>	> 500
<i>Aspergillus niger</i>	> 50
<i>Fusarium sp</i>	> 500
<i>Penicillium funiculosum</i>	> 50
<i>Aureobasidium pullulans</i>	> 50

Chemical name	3.3.8b. 1-[(Hydroxymethyl)amino]propan-2-ol
Chemical formula	C ₄ H ₁₁ NO ₂
Structural formula	CH ₃ -CH(OH)-CH ₂ -NHCH ₂ OH
Molecular mass	105.14
CAS-No.	176733-35-2
EC-No.	278-534-0
Synonym / common name	1-[(hydroxymethyl)amino]isopropanol, 2-hydroxypropyl-aminomethanol
Supplier	BAYER AG
Chemical and physical properties	
Appearance	clear, pale yellow liquid with an characteristic amine odour
Content%	100 (HCHO content 28–29)
Boiling point/range °C (101 kPa)	starts at approx. 100
Solidification point °C	–23
Density g/ml (20°C)	1.08
Vapour pressure hPa (20°C)	60
Viscosity mPas (20°C)	433
Flash point °C	> 100
Auto ignition temperature °C	approx. 320
pH (1% in water)	10.6
Log POW	–1.1
Stability	sensitive to acids; releases formaldehyde in aqueous media; on distillation condensation to 5-methyl-1,3-oxazolidine takes place
Solubility	highly soluble in H ₂ O and polar solvents
Toxicity data (source: BAYER AG)	
LD ₅₀ oral	1.430 mg/kg rat
Rabbit skin (exposure 4 h):	no irritation; corrosive to rabbit eyes. Sensitization may occur.
Ecotoxicity: Degradability	> 80% (closed bottle test). Activated sludge organisms are not inhibited by concentrations of 200 mg/l.
Incipient inhibition of cell multiplication in <i>Pseudomonas putida</i>	at 32 mg/l.
LC ₅₀ for <i>Brachydanio rerio</i> :	87 mg/l (96 h).

Antimicrobial effectiveness/applications

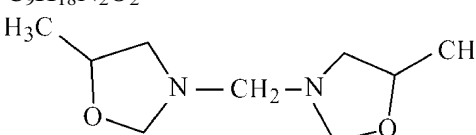
The antibacterial activity of N-(2-hydroxypropyl)-aminomethanol is jutting out, as is typical of most formaldehyde releasing compounds. The lowest MIC one needs for the suppression of sulphate reducing bacteria. In total the compound disposes of a broad spectrum of activity which takes effect between pH 4.5 to 12.

Accordingly N-(2-hydroxypropyl)-aminomethanol can be used for the in-can preservation of a great variety of aqueous products such as adhesives, bitumen emulsions, metalworking fluids. However, one has to keep in mind that formaldehyde releasing compounds are not in any case compatible with protein-based (e.g. casein-containing) products. Addition rates move between 0.05–0.3%.

Table 32 Minimum inhibition concentrations (MIC) of N-(2-hydroxypropyl)-aminomethanol in nutrient agar (Source: Bayer AG)

Test organisms	MIC in mg/l
<i>Aeromonas punctata</i>	275
<i>Bacillus subtilis</i>	750
<i>Desulfovibrio desulfuricans</i>	175
<i>Escherichia coli</i>	350
<i>Proteus vulgaris</i>	275
<i>Pseudomonas aeruginosa</i>	350
<i>Pseudomonas fluorescens</i>	350
<i>Staphylococcus aureus</i>	300
<i>Aspergillus flavus</i>	500
<i>Aspergillus niger</i>	600
<i>Aureobasidium pullulans</i>	600
<i>Chaetomium globosum</i>	1000
<i>Penicillium glaucum</i>	100
<i>Rhizopus nigricans</i>	500
<i>Candida krusei</i>	500
<i>Torula utilis</i>	500

Chemical name	3.3.8c. 2-[(Hydroxymethyl)amino]-2-methylpropanol
Chemical formula	$C_5H_{13}NO_2$
Structural formula	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{HO}-\text{CH}_2-\text{NH}-\text{C}-\text{CH}_2-\text{OH} \\ \\ \text{CH}_3 \end{array} $
Molecular mass	119.17
CAS-No.	see condensation product 3.3.10. =
EC-No.	4,4-dimethyl-1,3-oxazolidine
EPA-Reg.	approval for antimicrobial application
Synonym/common name	2-aminomethanol-2-methyl-propanol
Supplier	TROY
Chemical and physical properties	
Appearance	clear, pale amber liquid with a pungent odour
Content	75 (HCHO content 25)
Density g/ml (25°C)	0.980–1.010
Refractive index nD (20°C)	1.42–1.432
Flash point °C	52
pH value	approx. 11.2
Stability	sensitive to acids; releases formaldehyde in aqueous media; on distillation condensation to 4,4-dimethyl-1,3-oxazoline (3.3.10.) takes place; the product is not suitable for use in formulations with a pH < 7 and should not be exposed to temperatures exceeding 60°C
Solubility	highly soluble in water and polar solvents
Toxicity data/antimicrobial effectiveness/applications	
See the condensation product 3.3.10. regarding that this disposes of the higher formaldehyde content.	

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.9. 3, 3'-Methylenebis(5-methyl-1,3-oxazolidine)
Chemical formula	$C_9H_{18}N_2O_2$
Structural formula	
Molecular mass	186.26

CAS-No.	66204-44-2
EC-No.	266-235-8
Synonym/common name	bis-(5,5'-dimethyl-1,3-dioxazolidin-3-yl)-methane
Supplier	BODE CHEMIE, SCHUELKE & MAYR, SVENSKA SHELL

Chemical and physical properties

Appearance	nearly colourless fluid with a characteristic amine odour
Content (%)	approx. 100 (HCHO content approx. 48)
Boiling point/range °C (1,7 kPa)	116
Melting point °C	< 1
Density g/ml (20°C)	1.06
Vapour pressure hPa (20°C)	< 1
Flash point °C	> 100
Log POW (20°C)	-0.11
pH (1.5 g/l; 20°C)	approx. 10
Stability	sensitive to strong acids; releases formaldehyde in aqueous media, may be applicated at temperatures up to 80°C
Solubility	complete in H ₂ O; highly soluble in polar solvents

Toxicity data (source: SCHUELKE & MAYR)

LD ₅₀ oral	900 mg/kg rat
Irritant to skin and mucous membranes; 12.5% in water do not cause skin irritation; 0.2% in water are not irritant to the eyes.	

Ecotoxicity: The product is easily biodegradable (OECD method).

LC ₅₀ for <i>Brachydanio rerio</i>	57.7 mg/l (96 h).
EC ₅₀ for <i>Daphnia magna</i>	37.9 mg/l (48 h).
IC ₅₀ for <i>Scenedesmus subspicatus</i> (alga)	5.7 mg/l (72 h).
EC ₅₀ for bacteria	44 mg/l (OECD method 209).

Antimicrobial effectiveness/applications

Besides a broad spectrum of effectiveness (see Table 33) the methylenebisoxazolidine disposes of properties which with regard to its applications offer important advantages. Due to its alkalinity the microbicide is able to neutralize acids generated by proliferating micro-organisms; thus long lasting corrosion inhibition is supported. Remarkable is also the partition coefficient (log POW) avoiding the enrichment of the active ingredient in the

Table 33 Minimum inhibition concentrations (MIC) of 3,3'-methylenebis (5-methyl-1,3-oxazolidine) (Source: SCHUELKE & MAYR)

Test organism	MIC (% w/w)
Bacteria	
<i>Alcaligenes faecalis</i>	0.015
<i>Enterobacter cloacae</i>	0.030
<i>Escherichia coli</i>	0.030
<i>Proteus vulgaris</i>	0.030
<i>Pseudomonas aeruginosa</i>	0.030
<i>Pseudomonas fluorescens</i>	0.015
<i>Pseudomonas putida</i>	0.125
<i>Staphylococcus aureus</i>	0.030
<i>Sulphate reducing bacteria</i>	
<i>Desulfovibrio desulfuricans</i>	0.050
<i>Field isolation</i>	0.125
Fungi	
<i>Aspergillus niger</i>	0.030
<i>Fusarium oxysporum</i>	0.030
<i>Penicillium funiculosum</i>	0.015
Yeast	
<i>Candida albicans</i>	0.125
<i>Rhodotorula mucilaginosa</i>	0.030
<i>Saccharomyces cerevisiae</i>	0.125

organic phase of products to be protected from microbial growth in the water phase. Important application fields for the methylenebisoxazolidine are consequently fuel, metalworking fluids, drilling muds and other applications in oilfield operations.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

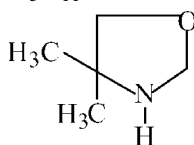
Chemical name

3.3.10. 4,4-Dimethyl-1,3-oxazolidine

Chemical formula

$C_5H_{11}NO$

Structural formula



Molecular mass

101.14

CAS-No.

51200-87-4

EC-No.

257-048-2; EEC-no. 45

EPA-Reg.

approval for antimicrobial applications

Synonym/common name

4,4-dimethyloxazolidine

Supplier

DEGUSSA, DOW-ANGUS, TROY

Chemical and physical properties

Appearance

clear, pale amber fluid with a pungent odour

Content%

77 (HCHO content approx. 21)

Boiling point/range °C (101 kPa)

99 (azeotrope)

Solidification point °C

< -20

Density g/ml (25°C)

0.985

Vapour pressure hPa (70°C)

133

Viscosity mPas (25°C)

approx. 7.5

Refractive index nD (20°C)

1.42–1.432

Flash point °C

49

pH (0.1 molar at 20°C)

11.0

Log POW

-0.14

Stability

sensitive to strong acids, releases formaldehyde at pH < 6 and in aqueous media; reacts with proteinaceous material completely in water; highly soluble in polar organic solvents

Solubility

Toxicity data (source: DOW-ANGUS)

LD₅₀ oral

956 mg/kg rat

dermal

1400 mg/kg rabbit

LC₅₀ inhalative (4 h)

2.48 mg/l for rats

Irritant to skin and mucous membranes. Not sensitizing in two guinea pig tests.

A mutagenic effect shown in vitro was not confirmed in vivo.

Subacute-chronic toxicity:

28-day oral application in the rat: NOEL 50 mg/kg/day.

90-day toxicity in the rat: no systemic toxicity at 195 mg/kg/day.

Teratogenicity study in rabbits, dermal application: no teratogenic effects at 300 mg/kg/day.

Ecotoxicity: Not easily biodegradable; degree of elimination 14% (closed bottle test).

LC₅₀ for rainbow trout

93 mg/l (96 h)

bluegill fish

46.1 mg/l (96 h)

golden orfe

50 mg/l (48 h)

LC₅₀ for daphnia magna

45 mg/l (48 h)

EC₀ for *Pseudomonas putida*

~30 mg/l

COD-value: 1480 mg/l

BOD5-value: 180–1400 mg/l

Antimicrobial effectiveness/applications

Due to its properties listed above 4,4-dimethyl-1,3-oxazolidine may be used for the in-can preservation of a great variety of water-based functional fluids, such as adhesives, cosmetics, drilling muds, fuels, polymer

emulsions, paints; this enumeration does not call for completeness. The addition rates move between 0.1 and 0.3%.

The MIC's listed in Table 34 show the breadth of the activity spectrum of the oxazolidine derivative. For each test organism is stated the highest concentration showing growth and the lowest concentration showing no growth.

Table 34 Minimum inhibition concentrations (MIC) of 4,4-dimethyl-1,3-oxazolidine (Source: DEGUSSA-CREANOVA)

Test organism	MIC (mg/l)
Bacteria	
<i>Bacillus megaterium</i>	125–250
<i>Bacillus mycoides</i>	125–250
<i>Bacillus subtilis</i>	125–250
<i>Desulfovibrio desulfuricans</i>	10–25
<i>Enterobacter aerogenes</i>	125–250
<i>Escherichia coli</i>	250–500
<i>Gafrkia tetragena</i>	125–250
<i>Lactobacillus acidiphilus</i>	62–125
<i>Micrococcus flavus</i>	62–125
<i>Mycobacterium ranae</i>	250–500
<i>Pasteurella multocida</i>	31–62
<i>Pasteurella pseudotuberculosis</i>	125–250
<i>Pseudomonas aeruginosa</i>	125–250
<i>Pseudomonas fluorescens</i>	250–500
<i>Proteus vulgaris</i>	125–250
<i>Salmonella typhii</i>	250–500m
<i>Shigella dysenteriae</i>	125–250
<i>Staphylococcus aureus</i>	125–250
<i>Streptococcus faecalis</i>	125–250
<i>Streptococcus hemolyticus</i>	250–500
Fungi	
<i>Aspergillus niger</i>	150–500
<i>Aspergillus fumigatus</i>	250–500
<i>Aureobasidium pullulans</i>	500–1000
<i>Candida albicans</i>	500–1000
<i>Cephalosporium sp.</i>	65–125
<i>Cladosporium herbarum</i>	500–1000
<i>Fusarium graminearum</i>	500–1000
<i>Fusarium moniliforme</i>	500–1000
<i>Penicillium sp.</i>	250–500
<i>Pullularia pullaris</i>	250–500
<i>Saccharomyces cerevisiae</i>	250–500
<i>Trichophyton mentagrophytes</i>	65–125

4,4-dimethyl-1,3-oxazolidine is effective in systems with pH values between 5 to 11. Using the microbicide one has to pay attention to the fact that the microbicide may increase or stabilize the pH of formulations into which it is incorporated.

In the EC list of preservatives allowed for the in-can protection of cosmetics 4,4-dimethyl-1,3-oxazolidine is mentioned with a maximum concentration of 0.1%, however only for products the pH of which is not lower than 6. This limitation is made, as at lower pH values 1,3-oxazolidines degrade to formaldehyde and the corresponding amines, the skin compatibility of which is limited.

If after the addition of 1M formaldehyde to 1M 1-amino-2-methyl-propanol from the resulting 2-(N-hydroxymethyl)-2-methyl-propanol 1 M H₂O is eliminated, the condensation reaction leads to 100% 4,4-dimethyl-1,3-oxazolidine which, compared with other amine-formaldehyde-reaction-products, where water is not completely eliminated and an equilibrium exists between the N-hydroxymethyl compound and the condensation product, has solubility properties favouring its incorporation in to oil concentrates (e.g. lubricoolants) and its application in fuels to inhibit microbial growth in fuel oil bottom water.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

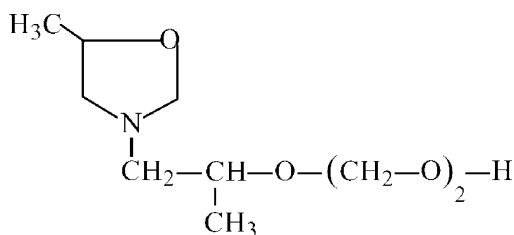
Chemical name

3.3.11. (5-Methyl-3-oxazolidinyl)-isopropanol-bishemiformal

Chemical formula

C₉H₁₉NO₄

Structural formula



Molecular mass

205.26

CAS-No.

220444-73-5

EC-Notification-no.

400

EPA-Reg.

approval for antimicrobial applications

Synonym/common name

[[[1-methyl-2-(5-methyl-3-oxazolidinyl)-ethoxy]methoxy]methoxy]methanol

Supplier

DEGUSSA-CREANOVA

Chemical and physical properties

Appearance

the addition/condensation product of 1M diisopropanolamine and 3M formaldehyde is a clear, colourless to light yellow liquid

Content (%)

50 (HCHO content ~22)

Solidification point °C

< -15

Density g/ml (25°C)

1.06-1.09

Flash point °C

60

pH

8.5-9.8

Stability

sensitive to acids; releases formaldehyde in aqueous solutions

Solubility

complete in water; highly soluble in organic solvents

Toxicity data

The toxicity of the microbicide is essentially specified by its content of formaldehyde (see 2.1.). The concentrated product causes irreversible eye damage or skin burns; it may be fatal if inhaled, harmful if swallowed or absorbed through skin.

Antimicrobial effectiveness/applications

The spectrum of effectiveness of this 1,3-oxazolidine derivative and its applications are comparable with those of other oxazolidine derivatives. The effectiveness is essentially appointed by the formaldehyde content of the compound. It will protect and preserve raw materials and finished products at use concentrations of 0.05 to 0.5% based on total weight of composition.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

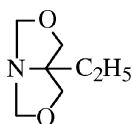
Chemical name

3.3.12. (5-Ethyl-3,7-dioxo-1-azabicyclo(3.3.0.)-octane

Chemical formula

C₇H₁₃NO₂

Structural formula



Molecular mass

143.19

CAS-No.

7747-35-5

EC-No.

231-810-4; EEC-no. 49

EPA-Reg.

approval for antimicrobial application

Synonym/common name

7-ethyl bicyclooxazolidine, 1-aza-3,7-dioxo-5-ethylbicyclo-(3.3.0.) octane
octane

Supplier

DOW-ANGUS

Chemical and physical properties

Appearance

pale, yellow liquid with a musty odour

Content%

97.5 (HCHO content ~42)

Boiling point/range °C (2.0 kPa)	71
Melting point °C	0
Density g/ml (20°C)	1.085
Vapour pressure hPa (71°C)	19.95
Viscosity mPa·s (25°C)	20.8
Surface tension mN/m (25°C)	36.5
Flash point °C	79.4
pH (0.1 M i. H ₂ O at 20°C)	10.2
Log POW	0.28
Stability	sensitive to acids, releases formaldehyde; compatible with cationic, anionic and nonionic surfactant systems over a pH range of 6–11
Solubility	soluble in water, ethanol, acetone, benzene, chlorinated hydrocarbons and mineral oil

Toxicity data (Source: DOW-ANGUS)

LD ₅₀ oral	> 3600 mg/kg rat
dermal	2000 mg/kg rabbit
LC ₅₀ on aerosol inhalation (4 h)	3.1 mg/l for rats

Irritant to skin, eyes and mucous membrane. Sensitization may occur.

In vitro mutagenicity tests were negative. NOEL observed in an oral teratology study with rats: 250 mg/kg/day. – NOEL for rats exposed dermally to the microbicide (5 days a week for 21 days): 100 mg/kg. – NOEL observed in a 28-day oral feeding study in rats: 100 mg/kg/day.

Ecotoxicity:

Biodegradation reached in Closed Bottle Test (OECD Method) after 28 days: 27%.

LC ₅₀ for rainbow trout	240 mg/l (96 h)
bluegill sun fish	130 mg/l (96 h)
pink shrimp	138 mg/l (96 h)
LC ₅₀ for <i>Daphnia magna</i>	42 mg/l (48 h)
EC ₅₀ for the algae <i>Scenedesmus</i>	15.7 mg/l (72 h)

Antimicrobial effectiveness/applications

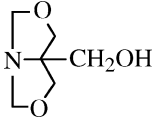
The broad antibacterial spectrum of 7-ethyl bicyclooxazolidine is demonstrated by the MIC's listed in Table 35. The minimum inhibitory ranges shown are the highest concentration showing growth and the lowest with no growth. The microbicide is successfully applied for the protection/preservation of a great variety of aqueous functional fluids, such as cosmetics, metalworking fluids. Addition rates: 0.05–0.3%. Worth mentioning are the alkaline buffering capability to prevent pH drift, the excellent thermal and alkaline stability, the partition

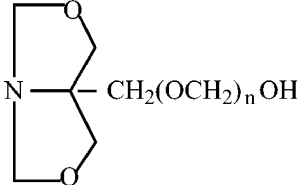
Table 35 Minimum inhibition concentrations (MIC) for 7-ethyl bicyclooxazolidine (Source: DOW-ANGUS)

Test organism	MIC (mg/l)
<i>Bacillus megaterium</i>	200–250
<i>Bacillus mycoides</i>	200–250
<i>Bacillus subtilis</i>	300–350
<i>Desulfovibrio aestuarii</i>	200–250
<i>Desulfovibrio desulfuricans</i>	150–200
<i>Enterobacter aerogenes</i>	250–300
<i>Escherichia coli</i>	450–500
<i>Gaffkya tetragena</i>	150–200
<i>Lactobacillus acidophilus</i>	200–250
<i>Micrococcus flavus</i>	100–150
<i>Micrococcus luteus</i>	450–500
<i>Proteus vulgaris</i>	300–350
<i>Pseudomonas aeruginosa</i>	800–850
<i>Pseudomonas fluorescens</i>	400–450
<i>Staphylococcus aureus</i>	200–250
<i>Streptococcus faecalis</i>	400–450
<i>Streptococcus hemolyticus</i>	400–450

coefficient which allows the partition of the active ingredient in the water and the oil phase of emulsions, the low odour level, the compatibility with a lot of particular formulation ingredients.

In the EC list of preservatives which cosmetic products may contain 7-ethyl bicyclooxazolidine is permitted with a max. addition rate of 0.3%, except in dental hygiene and mucous membrane contact products.

Microbicide group (substance class)	3.3 AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.13. 5-Hydroxymethyl-1-aza-3,7-dioxabicyclo(3.3.0) Octane
Chemical formula	$C_6H_{11}NO_3$
Structural formula	
Molecular mass	145.16
CAS-No.	59720-42-2
EC-No.	unknown
EPA-Reg.	approval for antimicrobial application
Synonym/common name	7-hydroxymethyl-bicyclic-oxazolidine
Chemical and physical properties	
Appearance	colourless crystals with a characteristic odour
Content (%)	~100 (HCHO content ~41)
Melting point °C	64
The addition of formaldehyde to the alcoholic hydroxy group leads to the formation of mono(poly)hemiformals of the bicyclic oxazolidine which constitutionally exhibit higher antimicrobial effectoness, as they are able to liberate more formaldehyde than the starting product. A mixture of such hemiformals which is in use as a microbicide is listed under 3.3.14.	

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.14 Polymethoxy Bicyclic Oxazolidines
Chemical formula	$C_7H_{13}NO_4$ (for $n = 1$)
Structural formula	
Molecular mass	175.17 (for $n = 1$)
CAS-No.	56709-13-8
EC-Notification-No.	394A(acceptable)
EPA-Reg.	approved for antimicrobial application
Synonym	5-hydroxypoly(methyleneoxy)-methyl-1-aza-3,7-dioxabicyclo(3.3.0) octane
Supplier	DEGUSSA-CREANOVA-COLORTREND
Composition of a formulation	24.5% 5-hydroxymethoxymethyl-1-aza-3,7-dioxabicyclo(3.3.0)octane 17.7% 5-hydroxymethyl-1-aza-3,7-dioxabicyclo(3.3.0)octane 7.8% 5-hydroxypoly(methyleneoxy)-methyl-1-aza-3,7-dioxabicyclo(3.3.0)octane ($n = 1$ 74%, $n = 2$ 21%, $n = 3$ 4%, $n = 4$ 1%)
Chemical and physical properties	
Appearance	light yellow fluid with a characteristic odour
Content (%)	50 (a.i.'s) (HCHO content ~24)
Boiling point/range °C (101 kPa)	100
Solidification point °C	-23.3
Density g/ml (20°C)	1.14

Vapour pressure hPa (20°C)	23
Refractive index nD (25°C)	1.404–1.412
Flash point °C	60
Auto ignition temperature °C	product is not self igniting
pH at 20°C	6–7.5
Stability	decomposes when heated (>100°C), sensitive to acids, releases formaldehyde
Solubility	complete in water, highly soluble in polar organic solvents
Toxicity data (source: DEGUSSA)	
LD ₅₀ oral	2.974 mg/kg rat
dermal	> 2.000 mg/kg rabbit
LD ₅₀ inhalative (4 h)	1.8–4.0 mg/l aerosol for rats
Irritant to skin, eyes and mucous membranes.	
May cause sensitization by skin contact.	

Antimicrobial effectiveness/applications

The MIC's listed in Table 36 are characteristic for a formaldehyde releasing compound: highly effective against bacteria, less effective against fungi. The preservative may be used in cosmetics and other aqueous functional fluids to protect against microbial activity during manufacture, storage and use. Optimum pH range: 5–9. Incorporation levels vary between 0.1 and 0.5% by weight.

Table 36 Minimum inhibition concentrations (MIC) of the formulation in nutrient agar (Source: DEGUSSA)

Test organism	MIC (mg/l)
Bacteria	
Gram Negative	
<i>Pseudomonas aeruginosa</i>	150
<i>Pseudomonas cepacia</i>	150
<i>Escherichia coli</i>	200
<i>Klebsiella ozaenae</i>	100
<i>Proteus vulgaris</i>	200
Gram Positive	
<i>Bacillus mycoides</i>	100
<i>Bacillus subtilis</i>	150
<i>Staphylococcus aureus</i>	150
Fungi	
Yeasts	
<i>Candida albicans</i>	1800
<i>Rhodoturula robra</i>	400
<i>Saccharomyces cerevisiae</i>	300
Molds	
<i>Aspergillus niger</i>	500
<i>Aspergillus oryzae</i>	500
<i>Aureobasidium pullulans</i>	250
<i>Helminthosporium gramineum</i>	400
<i>Penicillium funiculsum</i>	200
<i>Phoma pigmentivora</i>	600

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

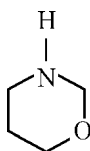
Chemical name

3.3.15. Tetrahydro-1,3-oxazine

Chemical formula



Structural formula



Molecular mass	87.12
CAS-No.	85367-17-5
EC-No.	unknown

Chemical and physical properties

Appearance	colourless fluid with a weak pungent odour
Content (%)	~100 (HCHO content 34)
Boiling point/range °C (101 kPa)	135–136
Reaction in H ₂ O	alkaline
Stability	relatively stable between pH values 6.5 and 10.5 degrades in acidic media quickly to the starting products
Solubility	highly soluble in water and polar solvents

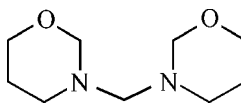
Tetrahydro-1,3-oxazines are formed by the addition of CH₂O to primary or secondary 3-hydroxy-alkylamines followed by intramolecular elimination of water, i. e. in analogy to the formation of 1,3-oxazolidines (Figure 13). Accordingly they behave similar, but do not present advantages. Due to their higher molecular weight they release a lower amount (%) of formaldehyde compared with the corresponding 1,3-oxazolidines. Tetrahydro-1,3-oxazines therefore have not much practical importance as preservatives for functional fluids.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name
Chemical formula
Structural formula

3.3.16. Bis-(tetrahydro-,3-oxazin-3-yl)methane
C₉H₁₈N₂O₂



Molecular mass
CAS-No.
EC-No.

186.25
63489-63-4
unknown

Chemical and physical properties

Appearance	colourless fluid with a weak odour
Content (%)	~100 (HCHO content 48)
Boiling point/range °C (1,5 kPa)	125
Reaction in H ₂ O	alkaline
Stability	stable in alkaline media, degrades in acidic media quickly to the starting products propanolamine and formaldehyde
Solubility	highly soluble in water and polar solvents

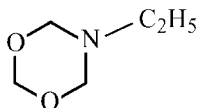
Although bis-(tetrahydro-1,3-oxazin-3-yl) methane is very effective according to its relatively high content of releasable formaldehyde and although it does not emit a pungent odour, it has not yet gained practical importance as a microbicide.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name
Chemical formula
Structural formula

3.3.17. N-ethyl-dihydro-1,3,5-dioxazine
C₅H₁₁NO₂



Molecular mass
CAS-No.
EC-No.

117.15
132292-80-9
unknown

Chemical and physical properties

Appearance	colourless fluid with a strong pungent odour
Content (%)	~100 (HCHO content 77)
Boiling point/range °C (5.5 kPa)	62–64
Reaction in H ₂ O	alkaline
Stability	of limited stability only, decomposes in water based systems to the starting products
Solubility	soluble in water and polar solvents

Dihydro-1,3,5-dioxazines are the reaction/condensation products of primary amines with 3 mol of formaldehyde. Although they release a higher quantity of formaldehyde than, for example, 1,3-oxazolidines and are therefore more effective than other formaldehyde releasing compounds they are not of practical importance as microbicides because of their limited stability and very pungent odour.

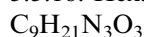
Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

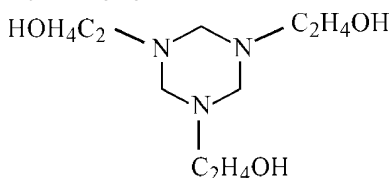
Chemical name

3.3.18. Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine

Chemical formula



Structural formula



Molecular mass

219.29

CAS-No.

4719-04-4

EC-No.

225-208-0

EPA-Reg.

approval for antimicrobial applications

Synonym/common name

2,2'2''-(hexahydro-1,3,5-triazin-1,3,5-triyl)triethanol, Hexahydrotriazine

Supplier

BASF, BODE, HENKEL, SCHUELKE & MAYR, THOR, TROY

Chemical and physical properties

Appearance	colourless to pale yellow clear fluid with a mild amine odour
Content %	~100 (HCHO content 41)
Boiling point/range °C (0.9 kPa)	100; the distillate is a fluid of low viscosity which rearranges exothermically to the viscous hexahydro-s-triazine (see Figures 10 and 13).
Solidification point °C	–20
Density g/ml (20°C)	1.157
Vapour pressure hPa (20°C)	25
Refractive index nD (20°C)	1.480–1.486
Flash point °C	> 100
Auto ignition temperature °C	250
Log POW	< 1
pH (1.5 g/l H ₂ O at 20°C)	10
Stability	sensitive to acids, stable over the pH range 7–12 and up to 80°C; releases formaldehyde in aqueous systems
Solubility	fully soluble in H ₂ O, highly soluble in most lower alcohols and glycols

Toxicity data (source: SCHUELKE & MAYR)

LD₅₀ oral

> 500–< 2000 mg/kg rat

dermal

> 2000 mg/kg rat

Non-mutagenic (micronucleus test).

Irritant to mucous membranes; slightly irritant to skin and eyes.

Ecotoxicity: Readily biodegradable (OECD-Test-Method).

Fish toxicity: LC₅₀ 27 mg/l.

Antimicrobial effectiveness/applications

The efficacy and the spectrum of effectiveness correspond to the amount of formaldehyde which may be released from the hexahydro-s-triazine derivative.

Table 37 Minimum inhibition concentrations (MIC) of hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	120
<i>Proteus mirabilis</i>	200
<i>Pseudomonas aeruginosa</i>	200
<i>Staphylococcus aureus</i>	200
Formaldehyde resistant bacteria	2500
<i>Candida albicans</i>	750
<i>Aspergillus niger</i>	800
<i>Penicillium glaucum</i>	150

The favourable solubility properties combined with low toxicity and good skin compatibility at the dilutions used allow the application of the compound in very different fields of application, mainly as a preservative for water-based functional fluids. Although the hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine is a formaldehyde releasing compound it is little pungent. Additionally it is cost effective. Therefore it is not surprising that the compound is one of the main preservatives for lubricoolants. Optimum pH range: 8-11.

Despite the figures for fungal inhibition, problems with fungal growth may occur in practical preservation situations as a result of under-dosing with respect to fungi (Rossmore & Holtzmann, 1974). Problems with formaldehyde resistant bacteria may occur, too, in consequence of the application of the hexahydro-s-triazine derivative alone (Paulus, 1976). These problems are hardly overcome by increasing the dosage as concentrations higher than 0.2% of a.i. are no longer cost effective and include the risk that formaldehyde is released in doses which are not tolerated at work places.

Microbicide group (substance class)

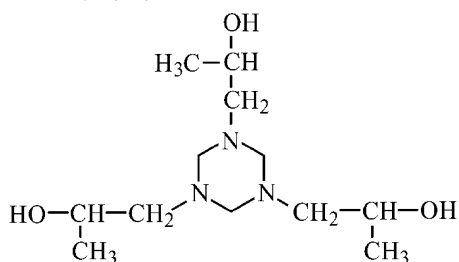
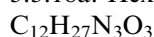
Chemical name

Chemical formula

Structural formula

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

3.3.18a. Hexahydro-1,3,5-tris(2-hydroxypropyl)-s-triazine



Molecular mass

CAS-No.

EC-No.

TSCA

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

Density g/ml (20°C)

Vapour pressure hPa (20°C)

Refractive index nD (20°C)

261.37

25254-50-56

246-764-0

listed

α,α',α'' -trimethyl-1,3,5-triazine-1,3,5(2H,4H, 6H)-triethanol

SCHÜLKE & MAYR

pale yellow fluid with a faint amine odour

approx. 80 (HCHO content approx. 27.55)

> 100

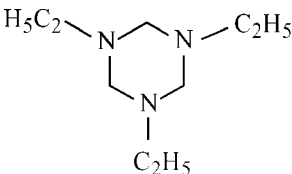
1.09

~25

1.47

Flash point °C	> 100
Auto ignition temperature °C	> 200
pH (2 g/l H ₂ O at 20°C)	~10

Further properties of the active agent, such as stability, solubility, toxicity, antimicrobial effectiveness and uses correspond to the homologous hexahydrotriazine derivative 3.3.18.

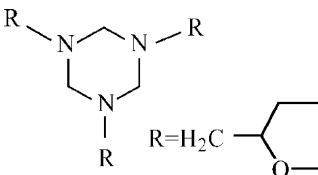
Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.19. Hexahydro-1,3,5-triethyl-s-triazine (HTT)
Chemical formula	C ₉ H ₂₁ N ₃
Structural formula	
Molecular mass	171.29
CAS-No.	7779-27-3
EC-No.	unknown
EPA-Reg.	approval for antimicrobial application
Synonym/common name	1,3,5-triethyl-1,3,5-hexahydro-triazine, triethyl-hexahydro-triazine
Supplier	R. T. VANDERBILT
Chemical and physical properties	
Appearance	colourless fluid with a strong to pungent amine odour
Content (%)	~100 (HCHO content 53)
Boiling point/range °C (101 kPa)	196–198
Refractive index nD (25°C)	1.4588
pH (0.5 g/l H ₂ O)	10.4
Stability	stable in alkaline media; degrades in acid formulations quickly to the starting products ethylamine and formaldehyde
Solubility	soluble in water, and in polar and non polar solvents, e.g. in petroleum distillate fractions (naphtha), kerosene, gas oil, mineral oils, propane, butane, toluene, xylenes, halogenated hydrocarbons

Toxicity data: Similar to those of 3.3.18

Antimicrobial effectiveness/applications

The spectrum of efficacy corresponds to that of the tris(2-hydroxyethyl)-derivative (3.3.18.), characterized by reduced fungicidal activity and a gap for 'formaldehyde resistant bacteria'. The latter are apparently identical with those which were identified as *Pseudomonas putida* by Hall & Eagon (1985) and given the strain designation 3-T-15². In the experiments of Barnes & Eagon (1986), neither HTT nor ethylamine nor CH₂O could be used by *P. putida* 3-T-15² as a carbon source for growth. However, according to Eagon & Barnes (1986) *P. putida* 3-T-15² has an active formaldehyde dehydrogenase. These findings are in line with those of Paulus (1976), who found that in a nutrient solution containing CH₂O, the latter is no longer detectable after incubation with 'formaldehyde resistant bacteria'.

Among the microbicides based on formaldehyde releasing compounds the hexahydro-s-triazine derivatives have the bulk of the market because of their cost effectiveness, compatibility and relative lack of toxicity. Main application field: metal working fluids (lubricoolants). Compared with the 2-hydroxyethyl compound (3.3.18.) the ethyl compound has a much more advantageous partition coefficient making it the triazine of choice for oil concentrates. Additionally its solubility in kerosene and related hydrocarbons make it a candidate for overcoming microbiological problems in jet fuels, heating fuel oils, etc., where there is a demand for microbicides combustible without any ash remaining. However, the pungent odour of HTT is detrimental to the microbicide in some application fields.

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.20. Hexahydro-1,3,5-tris[(tetrahydro-2-furanyl)-methyl]-s-triazine
Chemical formula	$C_{18}H_{33}N_3O_3$
Structural formula	
Molecular mass	339.48
CAS-No.	69141-51-1
EC-No.	unknown
Chemical and physical properties	
Appearance	yellow oil
Content (%)	~100 (HCHO content ~27)
Boiling point/range °C (0.1 kPa)	> 50
pH of aqueous solutions	alkaline
Stability	stable in alkaline media; degradation in acid formulations
Solubility	highly soluble in H ₂ O, alkanols, lipid like solvents, oil, lubricoolant concentrates
Toxicity data	
LD ₅₀ oral	1060 mg/kg mouse
subcutaneous	> 500 mg/kg mouse
intravenous	142 mg/kg mouse
Ames test: negative.	
A 5% w/v solution was not-irritant on the skin of rabbits (exposure: 24 h). A 1% solution caused slight irritation at the rabbit eye; a 0.1% solution was non-irritant.	

Antimicrobial effectiveness/applications

The minimum inhibition concentrations (MIC) for a broad spectrum of fungi associated with metal working fluid deterioration or of economic importance in industrial problems were determined by Grier et al. (1980) in

Table 38 Antifungal spectrum of hexahydro-s-triazines. (I = (tetrahydro-2-furanyl)-methyl-derivative; II – 2-hydroxyethyl-derivative)

Test fungi	MIC (mg/litre)		
	MF ^a	Triazine I	Triazine II
<i>Aspergillus niger</i>	442	300	200
<i>Alternaria solani</i>	3550	400	200
<i>Aureobasidium pullulans</i>	4341	200	100
<i>Botrytis alli</i>	3587	100	100
<i>Cephalosporium sp.</i>	4641	400	> 400
<i>Ceratocystis plifera</i>	4339	100	50
<i>Ceratocystis ulm</i>	4042	200	200
<i>Cladosporium fulvum</i>	35	100	100
<i>Cochliobolus miyabeanus</i>	4626	50	100
<i>Cochliobolus miyabeanus</i>	4630	50	100
<i>Fusarium oxysporum</i>	4014	400	400
<i>Fusarium sp.</i>	4642	> 400	> 400
<i>Helminthosporium biforme</i>	3640	400	300
<i>Helminthosporium cymodontis</i>	3642	100	100
<i>Penicillium digitatum</i>	4591	400	100
<i>Phoma sp.</i>	4332	100	100
<i>Scopulariopsis brevicaulis</i>	3769	100	100
<i>Pithomyces chartarum</i>	4395	50	100
<i>Trichoderma lignorum</i>	3560	300	300
<i>Trichoderma viride</i>	4064	200	50
<i>Ustilago zeae</i>	1996	100	50
<i>Verticillium albo-atrum</i>	3793	200	100
<i>Verticillium serrat</i>	3794	100	100

^a Merck fungi.

comparison to the corresponding MIC of hexahydro-1,3,5-tris (2-hydroxyethyl)-s-triazine (3.3.18.); see Table 38. Apparently there is no significant difference between the two hexahydro-s-triazinederivatives in their activity against fungi. Main application for the triazine I: preservation of industrial functional fluids, especially lubricoolants.

There is a significantly prolonged antifungal action obtained in lubricoolants when compared to failure with the tris(2-hydroxyethyl)-derivative although the MIC of the two hexahydro-s-triazines do not differ very much.

However, the fact that the (tetrahydro-2-furanyl)-methyl derivative is fivefold more lipoidal than the 2-hydroxyethyl analogue may be responsible for the effect (Grier et al., 1980).

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

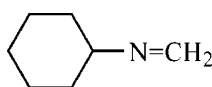
Solubility

Toxicity data: Not available.

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

3.3.21. N-Methylene-cyclohexylamine

$C_7H_{13}N$



111.19

4705-14-0

unknown

white, crystalline, faint amine odour

> 99 (HCHO content 27)

75

stable at pH values > 7; degradation in acid media (releases formaldehyde)

sparingly soluble in water, soluble in polar and non-polar solvents

Antimicrobial effectiveness/applications

The compound does not exhibit either special activity or cost effectiveness. It proved well in trials when used as a sanitizing agent in the dry cleaning process because of its good solubility in chlorinated hydrocarbons.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

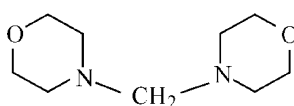
Content %

Boiling point/range °C (0.02 kPa)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

3.3.22. N,N'-methylene-bismorpholine

$C_9H_{18}N_2O_2$



186.26

5625-90-1

227-062-3

4,4'-methylene-bismorpholine, dimorpholino-methane

THOR

colourless, clear liquid with a mild amine odour

~100 (HCHO content 16)

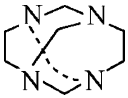
74

Density g/ml (20°C)	1.052
Refractive index nD (20°C)	1.479–1.483
pH (10% i. tapwater)	9.5–10.5
Amine value (mg/g)	585–605
Stability	stable over the pH range 8–12 and up to 100°C, degradation in acid media; not compatible with formaldehyde sensitive products, e.g. such containing proteins.
Solubility	complete in H ₂ O, most lower alcohols and glycols
Toxicity data: (source:THOR)	
LD ₅₀ oral	550 mg/kg rat
Irritant to the skin, mucous membranes and eyes. – Non-sensitizing.	
Non mutagenic according to Salmonella Microsome Test.	

Antimicrobial effectiveness/applications

The reaction of 1 mol formaldehyde with 2 mol secondary amine – in this case with morpholine – leads to amials, respectively diaminomethane derivatives (see Figure10).

When compared with other amine based formaldehyde releasing compounds dimorpholino-methane does not offer an advantage with respect to its antimicrobial activity, as the formaldehyde content (~16%) is relatively low. However, the microbicide is distinguished by favourable solubility properties and a weak odour, and accordingly suitable for the wet state preservation of a wide range of aqueous alkaline products such as metalworking fluids, cooling and lubricating emulsions, grinding fluids. Addition rates: 0.05–0.25%.

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.23. 1.4.6.9-Tetraaza-tricyclododecane (4.4.1.14.9)
Chemical formula	C ₈ H ₁₆ N ₄
Structural formula	
Molecular mass	168.24
CAS-No.	54159-21-6
EC-No.	unknown
Chemical and physical properties	
Appearance	white, odourless crystals
Content (%)	~100 (HCHO content 71)
Melting point °C	199
Reaction in H ₂ O	alkaline
Stability	sensitive to acids, releases formaldehyde
Solubility	very soluble in water, polar and non-polar solvents, e.g. in chlorinated hydrocarbons
Toxicity data not available	

Antimicrobial effectiveness/applications

Due to its high formaldehyde content the reaction product of ethylene diamine and CH₂O is one of the most effective formaldehyde-amine condensates. It may be produced as a 50% solution in water which is useful as

Table 39 Minimum inhibition concentrations (mg/litre) of 3.3.23

<i>Escherichia coli</i>	100
<i>Pseudomonas aeruginosa</i>	100
<i>Aspergillus niger</i>	1500
<i>Penicillium glaucum</i>	1000
<i>Rhizopus nigricans</i>	500

a preservative for aqueous functional fluids. Additionally the compound can serve as a sanitizing/disinfecting agent in dry cleaning fluids. Addition rates 0.05-0.15%.

Microbicide group (substance class)

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

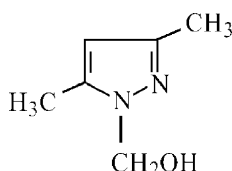
Chemical name

3.3.24. 3,5-Dimethyl-1-hydroxymethyl-pyrazole

Chemical formula

$C_6H_{10}N_2O$

Structural formula



Molecular mass

126.16

CAS-No.

85264-33-1

EC-No.

286-553-0

EPA-Reg.

for experimental use only

Synonym/common name

3,5-dimethyl-1H-pyrazole-1-methanol

Supplier

BUCKMAN

Chemical and physical properties

Appearance

white crystalline powder, as good as odourless

Content (%)

approx. 100 (HCHO content 23.8)

Melting point °C

110–113

Bulk density g/l (25°C)

493

Vapour pressure hPa (20°C)

0.0034

Stability

stable to 100°C; releases formaldehyde in aqueous formulations

Solubility (g/l)

42 in H_2O , 200 in propylene glycol

Toxicity data: (source: Buckman)

LD₅₀ oral

2.600 mg/kg rat

dermal

> 2.000 mg/kg rabbit

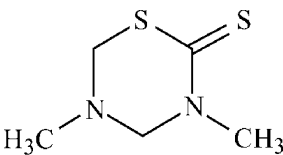
Irritant to skin and eyes (rabbit test). No evidence of sensitization in the guinea pig test. – Not a mutagen (Ames test negative).

Antimicrobial effectiveness/applications

As a *N*-methylol-pyrazole derivative the substance releases formaldehyde which is quantitatively detectable by the Tanenbaum method; in consequence the antimicrobial effectiveness of the compound corresponds to its formaldehyde content. It may be used for the in-can/in-tank protection of water based functional fluids, such as latex paints, adhesives, lubricoolants, polymer emulsions, detergents, cosmetics; it is effective over a broad pH range (3–11, 5).

Table 40 Minimum inhibition concentrations (MIC) of 3,5-dimethyl-2-hydroxymethyl-pyrazole in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	> 800
<i>Chaetomium globosum</i>	400
<i>Scopulariopsis brevicaulis</i>	> 800
<i>Lentinus tigrinus</i>	800
<i>Pseudomonas aeruginosa</i>	800
<i>Staphylococcus aureus</i>	400

Microbicide group (substance class)	3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.3.25. Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
Chemical formula	$C_5H_{10}N_2S_2$
Structural formula	
Molecular mass	162.28
CAS-No.	533-74-4
EC-No.	208-576-7
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	Dazomet
Supplier	BASF, BUCKMAN, CLARIANT-NIPA, DEGUSSA-CREANOVA-COLOR TREND, HENKEL, THOR
Chemical and physical properties	
The microbicide is listed here under the amine-formaldehyde-reaction-products, because it is synthesized by condensation of formaldehyde with methyl amine and carbon disulphide.	
Appearance	white crystalline powder or granulate forms with a faint sulfurous odour
Content %	98–100 (HCHO content 37)
Melting point °C	104–105 (decomposition)
Bulk density kg/l (20°C)	1.2–1.4/0.350
Vapour pressure hPa (20°C)	0.4
Flash point °C	> 360
Auto ignition temperature °C	product is not self igniting
Stability	stable at temperatures up to 35°C, sensitive to moisture; hydrolysis to methyl isothiocyanate (20.9.2.), methyl amine, formaldehyde (2.1.) and H ₂ S
Solubility g/kg (20°C)	3 in H ₂ O, 15 in ethanol, 173 in acetone, 391 in chloroform, 51 in benzene, 0.4 in cyclohexane
Toxicity data: (source: BASF)	
LD50 oral	approx. 500 mg/kg rat
dermal	> 2000 mg/kg rat
Not irritant on the skin of rabbits (OECD 404).	
Not irritant on the eyes of rabbits (OECD 405).	
Ecotoxicity: Extremely hazardous for water (hydrolysis to methyl isothiocyanate)	
EC ₅₀ for bacteria (17 h)	1–10 mg/l
LC ₅₀ for fish (<i>Onchorynchus mykiss</i>)	0.1–1 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	0.1–1 mg/l (48 h)

Antimicrobial effectiveness/applications

As the MIC in Table 41 prove, Dazomet has an extraordinary broad spectrum of high activity which covers bacteria (including “formaldehyde resistant bacteria” – MIC 200 mg/l), fungi and yeasts, indicating that the substance is a very special formaldehyde releasing compound. One finds an explanation by looking to the fact that hydrolysis of Dazomet in neutral to alkaline media leads to a highly reactive intermediate, namely to methyl isothiocyanate (20.9.2.) which exceeds the also released formaldehyde in antimicrobial effectiveness and breadth of activity. As Dazomet kills soil fungi, nematodes and soil insects, too, it may be used also for the treatment of soil. According to that it has to be regarded as a biocide; that it is more toxic and ecotoxic than formaldehyde is not surprising.

Table 41 Minimum inhibition concentrations (MIC) of Dazomet (Source: BASF)

Test organism		MIC (mg/l)
Gram positive bacteria		
<i>Staphylococcus aureus</i>	ATCC 6538	117
<i>Bacillus cereus</i>	NCTC 2599	117
<i>Bacillus subtilis</i>	NCTC 10073	117
<i>Thiobacillus thioparus</i>	NCIMB 8370	2
Gram negative bacteria		
<i>Pseudomonas aeruginosa</i>	NCIMB 8626	117
<i>Burkholderia cepacia</i>	NCIMB 9085	78
<i>Pseudomonas fluorescens</i>	NCIB 9046	234
<i>Escherichia coli</i>	NCIMB 8545	375
<i>Klebsiella aerogenes</i>	NCTC 418	375
<i>Klebsiella pneumoniae</i>	PC 1602	117
<i>Enterobacter aerogenes</i>	NCTC 10006	234
Sulphate reducing bacteria		
<i>Desulphovibrio desulphuricans</i> (Marine species)	NCIB 8314	2
<i>Desulphovibrio desulphuricans</i> (Freshwater species)	NCIB 8307	150
Yeasts		
<i>Candida albicans</i>	NCPF 3179	375
<i>Saccharomyces cerevisiae</i>	NCYC 87	375
Moulds		
<i>Aspergillus niger</i>	IMI 149007	188
<i>Trichoderma viridae</i>	(Industrial isolate)	375
<i>Penicillium funiculosum</i>	IMI 87160	47
<i>Stachybotrys atra</i>	IMI 82021	94

Dazomet's spectrum of activity is attractive for application of the compound in a number of industrial systems, e.g. as a slimicide in closed water circuits, paper machine systems, as a broad spectrum microbicide which prevents fungal blooms in metalworking fluid systems, or is suitable for the wet state preservation of a wide range of formulations, products for which the incorporation of a powder microbicide is necessary included. Compatibility with anionic, cationic and non-ionic surfactants increases formulation opportunities and improves effectiveness. However, there are limitations: poor water solubility, instability, release of H₂S and accordingly coloration by reaction with heavy metal salts.

A pH between 4 and 9 is the optimum for Dazomet. The addition rates move between 0.01 and 0.1%.

Microbicide group (substance class)

Chemical name

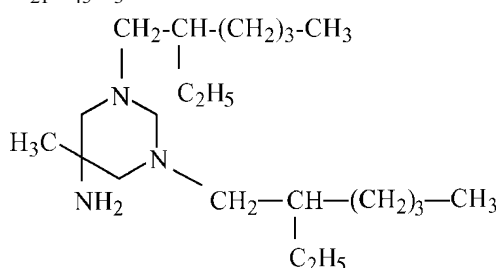
Chemical formula

Structural formula

3.3. AMINE-FORMALDEHYDE-REACTION-PRODUCTS

3.3.26. 5-Amino-1,3-bis(2-ethylhexyl)-5-methyl-hexahydro-pyrimidine

C₂₁H₄₅N₃



Molecular mass

339.61

CAS-No.

141-94-6

EC-No.

205-513-5; EEC-no. 19

Synonym/common name

1,3-bis(2-ethylhexyl)-5-methyl-hexahydropyrimidin-5-yl-amine, Hexetidine

Supplier

DOW-ANGUS

Chemical and physical properties

Appearance

colourless viscous fluid with a faint amine odour

Content (%)

~100 (HCHO content 27)

Boiling point/range °C (0.05 kPa)	160
Density g/ml (20°C)	0.89
Refractive index nD (20°C)	1.4640
Stability	comparatively heat resistant; does not separate formaldehyde under condition of use
Solubility	sparingly in water (0.01%), soluble in ethanol, methanol, acetone
Toxicity data	
LD ₅₀ oral	1.430 mg/kg rat
intrapertitoneal	30–85 mg/kg mouse
dermal	1.86 ml/kg rat
	> 4.0 ml/kg rabbit
Irritant to skin and mucous membranes; 0.5% solutions proved to be non-irritant.	
Sensitization is not observed.	
Ames test: negative.	

Antimicrobial effectiveness/applications

From the spectrum of effectiveness one can conclude that the activity of Hexetidine is not the result of released formaldehyde. This is in line with the fact that the formaldehyde contained in Hexetidine is not traceable with the Tanenbaum method or other more severe methods, although the synthesis pathway for Hexetidine starts with the reaction of 2-ethylhexylamine and nitroethane with formaldehyde. The intermediate (substituted 5-nitro-hexahydro-pyrimidine) is reduced to Hexetidine. Apparently Hexetidine is effective as a membrane active substance which is inactivated by acids and soaps. Non-ionic detergents can support the activity of Hexetidine. One has to pay attention to the deep gaps in the spectrum of effectiveness with regard to Pseudomonads. The extraordinary high activity of Hexetidine against fungi is remarkable. Optimum pH range 4–7.

Hexetidine is used as a microbicide in cosmetics and in pharmaceutical products. In the EC positive list of preservatives which cosmetics may contain it is mentioned with a maximum authorized concentration of 0.1%.

The gaps in the spectrum of effectiveness of Hexetidine have to be closed by combination with other suitable microbicides.

Table 42 Minimum inhibition concentrations (MIC) of Hexetidine in nutrient agar

Test organism	MIC (mg/litre)
<i>Staphylococcus aureus</i>	5–10
<i>Escherichia coli</i>	1250
<i>Pseudomonas aeruginosa</i>	> 5000
<i>Candida albicans</i>	5000
<i>Aspergillus niger</i>	800
<i>Chaetomium globosum</i>	75
<i>Penicillium brevicaulis</i>	400

3.4. Amide-formaldehyde-reaction-products

When heated under neutral to weakly alkaline conditions amides react quantitatively with formaldehyde to give *N*-hydroxymethyl amides (R-CO-NH-CH₂OH). These have a higher degree of stability than *N*-hydroxymethyl amines or their condensation products (3.3.). The formaldehyde releasable from *N*-hydroxymethyl amides is not detectable using the Tanenbaum method. As a result *N*-hydroxymethyl amides generally fail to produce substantial antimicrobial activity. That is the price for the higher degree of stability.

However, an exception must be made for those *N*-hydroxymethyl amides that derive from antimicrobial effective compounds having an amide configuration in the molecule, e.g. α -halogen-amides (introduction of a second toxophoric group into the molecule).

At first sight the effectiveness of these amides does not seem to be improved, but rather reduced by the introduction of the hydroxymethyl group. But in practice, on using the substances as preservatives in neutral to alkaline, aqueous functional fluids, a partial splitting into formaldehyde and the starting compound will be observed (see Figure 14), the antimicrobial effects of the breakdown products complementing each other in such a way that the effective spectrum is broadened. Finally, this is the effect of substances bearing two toxophoric groups or structural elements in one molecule.

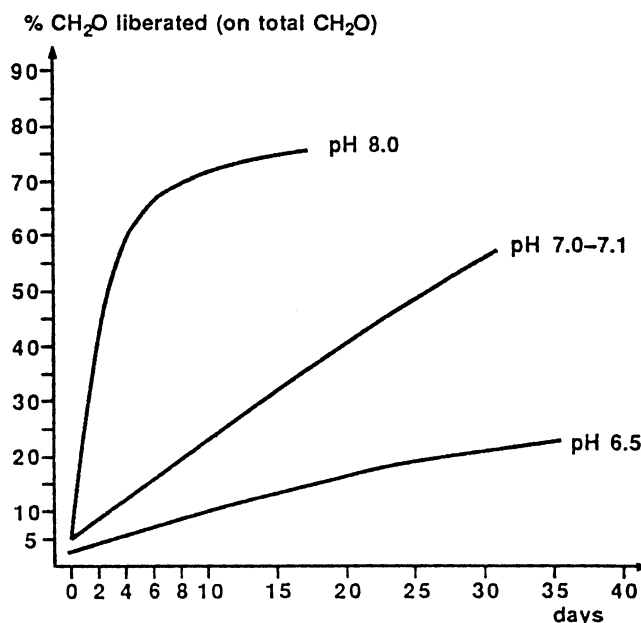


Figure 14 Separation of CH_2O in 0.5% solutions of *N*-hydroxymethyl-chloracetamide in water at 20°C with different pH values. CH_2O released determined by the method of Tanenbaum.

Amides derive also from carbonic acid. The hemiamide of carbonic acid = carbamic acid ($\text{H}_2\text{N}-\text{COOH}$) cannot be in existence; it degrades spontaneously to CO_2 and NH_3 . However, salts and esters of carbamic acid are more or less stable. The esters, $\text{H}_2\text{N}-\text{COOR}$, are named urethanes. They react with ammonia to urea, the diamide of carbonic acid, $(\text{NH}_2)_2\text{C}=\text{O}$. Urethanes and urea add readily formaldehyde forming *N*-hydroxymethyl or *N,N'*-bis-hydroxymethyl derivatives which are formaldehyde releasing compounds.

Carbondisulfide (CS_2) reacts in analogy to CO_2 with amines to dithiocarbamates ($\text{H}_2\text{N}-\text{CSSR}$) (see 11. Carbamates) and thiourea $(\text{NH}_2)_2\text{C}=\text{S}$. The addition of formaldehyde leads to *N*- or *S*-hydroxymethyl derivatives, formaldehyde liberating substances.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

3.4.1. *N*-Hydroxymethyl-chloracetamide

$\text{C}_3\text{H}_6\text{ClNO}_2$

$\text{Cl}-\text{CH}_2-\text{CO}-\text{NH}-\text{CH}_2-\text{OH}$

123.54

2832-19-1

220-598-9

N-methlol-chloracetamide, MCA

SCHUELKE & MAYER

Chemical and physical properties

Appearance

Content (%)

Melting point $^\circ\text{C}$

Reaction in water

Stability

white, odourless crystals

approx. 100 (HCHO content 24)

102

neutral

releases formaldehyde in neutral to alkaline media; formaldehyde is not detectable under the conditions of the Tanenbaum analysis method; chloride ions split off at pH values > 9

Solubility g/l (20°C)

260 in H_2O , 200 in methanol, sparingly soluble in non-polar solvents

Toxicity data

LD ₅₀ oral	340 mg/kg rat
dermal	> 500 mg/kg rat (exposure: 7 days)

Slightly irritant to skin and mucous membranes.

Antimicrobial effectiveness/applications

MCA's efficacy increases with pH; the optimum activity is achieved between pH 7.5 and 9. This is due to the fact that the amount of formaldehyde liberated from MCA increases with pH (see Figure 14).

The MIC in Table 43 allow the following conclusions: In slightly acidic media chloroacetamide (CA, 17.1.), in comparison to MCA, is the more active microbicide. However, at pH 8, where MCA releases approx. 75% of its formaldehyde, MCA is more effective, as the breakdown products (CA and CH₂O) now complement each other. MCA is an ideal preservative for the protection of technical functional fluids having a slightly alkaline pH, e.g. polymer emulsions, water based paints, adhesives. For all that the good water solubility and compatibility with many different aqueous formulations is an important advantage. Besides that a favourable partition coefficient guarantees that MCA remains in the water phase of two phase systems, thus attacking the microbes in the phase where they are vegetating. Colourlessness and odourlessness complete the advantageous properties of MCA as a preservative.

Generally the activity of formaldehyde releasing compounds is reduced in alkaline media containing ammonia, because of the formation of inactive hexamethylene tetramine (3.3.1.). In the case of MCA, however, the hexamethylenetetramine formed reacts with MCA or CA to give quaternary hexaminium salts (3.3.5. or 3.3.6) which are effective, as they release formaldehyde widely independent of pH (see 3.3).

However, altogether the importance of MCA as a preservative for water-based functional fluids is on the decline, as there is no great hope of the toxicological profile.

Table 43 Minimum inhibition concentrations (MIC) of MCA and chloroacetamide (CA) at different pH values in nutrient agar

Test organism	MIC (mg/litre)			
	MCA		CA	
	pH 5	pH 8	pH 5	pH 8
Escherichia coli	> 2500	1000	> 2500	> 2500
Pseudomonas aeruginosa	1500	1200	1000	1200
Staphylococcus aureus	> 2500	600	800	2500
Aspergillus niger	4000	1500	1000	1000
Chaetomium globosum	1000	750	500	500
Penicillium glaucum	4000	1000	500	750
Rhizopus nigricans	4000	750	500	500

Microbicide group (substance class)

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

Chemical name

3.4.2. 2,2,3-Trichloro-N-hydroxymethyl-propionamide

Chemical formula



Structural formula



Molecular mass

206.46

CAS-No.

7321-41-7

EC-No.

unknown

Chemical and physical properties

Appearance

white, odourless crystals

Content (%)

approx. 100(HCHO content 15)

Melting point °C

98

Reaction in water

neutral

Stability

releases formaldehyde in neutral to alkaline media; the compound's formaldehyde content is not detectable under the conditions of the Tanenbaum analysis method; chloride ions split off at pH values > 9

Solubility

soluble in H₂O, polar and non-polar solvents

Toxicity data not available

Antimicrobial effectiveness/applications

Compared with MCA (3.4.1.) 2,2,3-trichloro-N-hydroxymethylpropionamide does not exhibit special activity. However, because of its excellent solubility properties – soluble not only in water but in non-polar solvents, too- it has been characterised by Paulus et al., 1967 as an active ingredient which is highly appropriated as a preservative for oil-based lubricoolants.

Microbicide group (substance class) 3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name 3.4.3. N-hydroxymethyl-ureas

<i>Compound</i>	<i>Structural formula</i>	<i>Empirical formula</i>	<i>M_r</i>	<i>CH₂O content</i>	<i>Appearance</i>	<i>CAS-No.</i>	<i>EC-No.</i>
N-hydroxymethyl-urea	$\begin{array}{c} \text{HN-CO-NH}_2 \\ \\ \text{CH}_2\text{OH} \end{array}$	C ₂ H ₆ N ₂ O ₂	90.08	33%	Solid, m.p. 111°C	1000-82-4	213-674-8
N, N'-bis-hydroxymethyl-urea	$\begin{array}{c} \text{HN-CO-NH} \\ \quad \\ \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \end{array}$	C ₃ H ₈ N ₂ O ₃	120.11	50%	Solid, m.p. 126°C	140-95-4	205-444-0

The hydroxymethyl ureas are colourless, odourless and distinguished by good water solubility; they release formaldehyde in water based solutions, especially in alkaline solutions, and therefore have been used to some extent as preservatives in alkaline functional fluids, e.g. lubricoolants. In more neutral solutions the hydroxymethyl ureas release a small amount only of their formaldehyde content. Using hydroxymethyl ureas as preservatives one has to bear in mind that a nutrient for microbes is left, when the formaldehyde is separated and used up by irreversible reactions with other compounds or microorganisms.

Microbicide group (substance class)

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

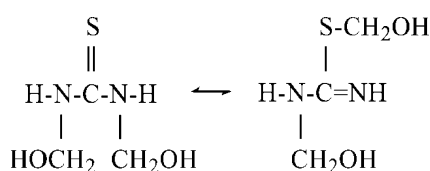
Chemical name

3.4.4. N,N'-bis(hydroxymethyl)thiourea <—> N-hydroxymethyl-S-hydroxymethyl-thiourea

Chemical formula

C₃H₈N₂O₂S

Structural formula



Molecular mass

136.18

CAS-No.

3084-25-1

EC-No.

unknown

Synonym/common name

dimethylol thiourea

Chemical and physical properties

Appearance

white, crystalline solid

Content (%)

~100 (HCHO content 44)

Melting point °C	~86
Stability	tolerates heating to 80°C; releases formaldehyde in water-based media
Solubility	soluble in H ₂ O and alcohols

Both tautomeric forms of thiourea can react with formaldehyde. The S-hydroxymethyl isomers are formed as the principal products under alkaline conditions; however, it is not believed that the materials obtained are 100% isomerically pure (Walker, 1975).

Antimicrobial effectiveness/applications

The efficacy is in line with the formaldehyde separated and covers bacteria, yeasts and fungi accordingly. The compound only occasionally has been used as a preservative in functional fluids.

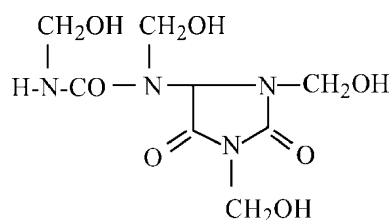
Microbicide group (substance class)	3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.4.5. N-hydroxymethyl-N'-methyl-thiourea
Chemical formula	C ₃ H ₈ N ₂ OS
Structural formula	$ \begin{array}{c} \text{H} \quad \text{S} \quad \text{H} \\ \diagdown \quad \parallel \quad \diagup \\ \text{N}-\text{C}-\text{N} \\ \diagup \quad \quad \diagdown \\ \text{H}_3\text{C} \quad \quad \text{CH}_2\text{OH} \end{array} $
Molecular mass	120.18
CAS-No.	15599-39-0
EC-No.	unknown
Chemical and physical properties	
Appearance	white, crystalline powder
Content (%)	~100 (HCHO content 25)
Melting point °C	84–86
Stability	heat resistant to 80°C; sensitive to light; separates formaldehyde slowly in water-based formulations: in a 0.5% solution 0.035% HCHO are detectable after 4 days by the Tanenbaum method, i.e. 30% of the total HCHO content
Solubility g/l (20°C)	100 in H ₂ O, soluble in ethanol
Toxicity data	
LD ₅₀ oral	> 3.000 mg/kg mouse
subcutaneous	~1.600 mg/kg mouse

Antimicrobial effectiveness/applications

To underline is the efficacy against Pseudomonads. However up to now the substance is only occasionally used as a microbicide.

Microbicide group (substance class)	3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.4.6. N-(hydroxymethyl)-N-[1,3-bis(hydroxymethyl)-2,5-dioxo-imidazolidin-4-yl]-N'-hydroxymethyl-urea.
Chemical formula	C ₈ H ₁₄ N ₄ O ₇

Structural formula



Molecular mass

278.23

CAS-No.

78491-02-8

EC-No.

278-928-2; EEC-no. 46

Synonym/common name

Diazolidinyl urea

Supplier

CLARIANT-NIPA, SUTTON LAB.

Chemical and physical properties

Appearance

white, crystalline powder with a slight odour

Content (%)

~100 (HCHO content 43)

Melting point °C

~150 (decomposition)

Reaction in water

neutral

Stability

highly hygroscopic, stable up to 60°C; the release of formaldehyde in water-based formulations occurs very slowly

Solubility g/l (20°C)

700 in H₂O, 0.1 in ethanol, 360 in glycerol, 400 in propylene glycol

Toxicity data

LD₅₀ oral

2570 mg/kg rat

dermal

> 2000 mg/kg rabbit

1% and 5% solutions are not irritant to skin and mucous membranes. A slight sensitization only is observed in the guinea pig test.

Antimicrobial effectiveness/applications

The efficacy of diazolidinyl urea is mainly a bacteriostatic one because of the slow release of formaldehyde. The addition rates which are generally applied (0.1–0.3%) are not effective against mould producing fungi and yeasts. However, because of its favourable toxicological data diazolidinyl urea is often used as a preservative for cosmetics. Accordingly it is listed in the corresponding EC positive list (maximum authorized concentrations: 0.5%). Percentage of use in US cosmetic formulations: 1.40%. The spectrum of effectiveness may be completed by using diazolidinyl urea in combination with *p*-hydroxy-benzoates (8.1.11.); such combinations exhibit a marked synergism (Berke & Rosen, 1982a). Diazolidinyl urea is effective over a wide pH range (3.0–8.5) and retains its activity in the presence of proteins and nonionic surfactants and other common ingredients of personal care products.

Table 44 Minimum inhibition concentration (MIC) of diazolidinyl urea (Source: CLARIANT-NIPA)

Test organism		MIC in %
<i>Pseudomonas aeruginosa</i>	NCIB 8626	0.10
<i>Pseudomonas putida</i>	NCTC 10936	0.15
<i>Escherichia coli</i>	NCIB 8545	0.125
<i>Proteus vulgaris</i>	NCTC 4635	0.125
<i>Salmonella enteritidis</i>	NCTC 5188	0.15
<i>Staphylococcus aureus</i>	ATCC 6538	0.08
<i>Bacillus cereus</i>	NCTC 7464	0.10
<i>Bacillus subtilis</i>	NCTC 3160	0.10
<i>Enterococcus faecium</i>	DVG 8582	0.10
<i>Streptococcus pyogenes</i>	ATCC 19615	0.25
<i>Candida albicans</i>	NCPF 3179	> 0.60
<i>Saccharomyces cerevisiae</i>	NCYC 200	0.15
<i>Aspergillus niger</i>	IMI 149007	0.30
<i>Penicillium purpurogenum</i>	Nipa Stock D15	0.15

permitted for the in-can protection of cosmetics imidazolidinyl urea is mentioned with a maximum authorized concentration of 0.6%. Percentage of use in US cosmetic formulations: 13.72%. Combinations of imidazolidinyl urea with p-hydroxybenzoates (8.1.11.), e.g. 0.3–0.5% imidazolidinyl urea with 0.2% p-hydroxymethylbenzoate and 0.1% p-hydroxypropylbenzoate, have proved a success in killing the most resistant strains of *Pseudomonads*, and additionally are able to overcome the limited antifungal activity of imidazolidinyl urea.

The ability of imidazolidinyl urea to be active over a wide pH range is as remarkable as its compatibility with almost all cosmetic ingredients.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

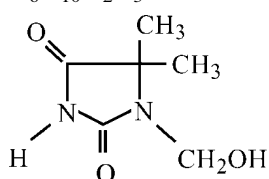
EC-No.

Synonym/common name

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

3.4.8. 1-(Hydroxymethyl)-5,5-dimethyl-2,4-dioxo-imidazolidine

$C_6H_{10}N_2O_3$



158.16

28453-33-0

240-352-4

Monohydroxymethyl-5,5-dimethyl-hydantoin, MDMH

Chemical and physical properties:

2,4-Dioxo-imidazolidines, also called hydantoins, include the carbonamide structure, too, and therefore the corresponding N-hydroxymethyl compounds here are listed among the reaction products of amides and formaldehyde. The monohydroxymethyl compound is described for the sake of completeness; the more active and important microbicide is the dihydroxymethyl compound 3.4.9.

Appearance

Content (%)

Melting point °C

Reaction in water

Stability

white, crystalline, odourless solid

~100 (HCHO content 19)

~100

neutral

stable up to 85°C; releases formaldehyde in aqueous solutions of pH 6; a part only (approx. 30%) of the formaldehyde content is detectable under the conditions of the Tanenbaum analysis method

Antimicrobial effectiveness/applications

The monohydroxymethyl-dimethyl-hydantoin produces a spectrum of activity which is characteristic of formaldehyde releasing compounds. The microbicide is proposed for the preservation of cosmetic and pharmaceutical products; addition rate 0.2%.

Microbicide group (substance class)

Chemical name

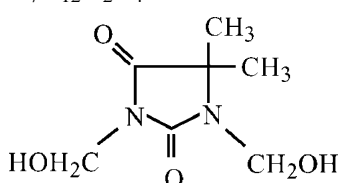
Chemical formula

Structural formula

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

3.4.9. 1,3-Bis(hydroxymethyl)-5,5-dimethyl-2,4-dioxo-imidazolidine

$C_7H_{12}N_2O_4$



Molecular mass	188.19
CAS-No.	6440-58-0
EC-No.	229-228-8; EEC-no. 33
USA	recognition by CTFA.
Synonym/common name	N,N'-dihydroxymethyl-5,5-dimethyl-hydantoin, DMDMH
Supplier	CLARIANT-NIPA, LONZA, TROY

Chemical and physical properties of a 55% equilibrium mixture in water

Appearance	clear, nearly colourless fluid with a faint odour of formaldehyde; water content $45 \pm 1\%$
Content (%)	~ 55 (total HCHO 18 ± 1 ; free HCHO 1 max)
Freezing point °C	-11 ± 0.6
Density g/ml (25°C)	1.16
Flash point °C	> 100
pH at 20°C	6.5–7.5
Stability	stable between pH 6 and 8; 1–2% of the HCHO content are detectable by the Tanenbaum method; release of HCHO increases with increase of pH, DMDMH tolerates heating to 80°C; it is fully compatible with cationic, anionic or non-ionic surfactants, emulsifiers and proteins completely in water
Solubility	

Toxicity data (source: LONZA)

LD ₅₀ oral	1.6903 mg/kg rat

Mild irritant to the skin (test with rabbits, exposure 24 h).

Skin, eye ingestion and sensitization studies clearly indicate DMDMH is safe and presents minimal hazards at recommended use levels.

Ecotoxicity:

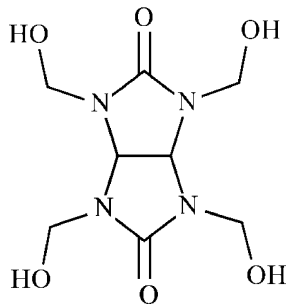
LC ₅₀ for rainbow trout	283 mg/l (96h)
bluegill sunfish	95 mg/l (96 h)
EC ₅₀ for Daphnia magna	20.4 mg/l (48 h)

Antimicrobial effectiveness/applications

DMDMH's effectiveness over wide pH and temperature ranges bases on the release of HCHO. The activity and spectrum of effectiveness is shown by the MIC's in Table 46. The favourable chemical and physical properties of the active ingredient and its low toxicity led to its inclusion into the EEC list of preservatives which cosmetic products may contain; max. accepted concentration: 0.6% (100% a. i.). Percentage of use in US cosmetic formulations: 2.75%.

Table 46 Minimum inhibition concentrations (MIC) of the DMDMH solution (Source: LONZA)

Test organism	MIC (mg/l)
Escherichia coli	291
Staphylococcus aureus	291
Pseudomonas aeruginosa	291
Pseudomonas cepacia	727
Bacillus subtilis	727
Enterobacter cloacae	1455
Streptococcus pyogenes	364
Sarcina lutea	364
Serratia marcescens	727
Saccharomyces cerevisiae	1455
Aspergillus niger	1455
Trychophyton mentagrophytes	727

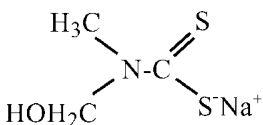
Microbicide group (substance class)	3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.4.10. 1,3,4,6-Tetrakis-hydroxymethyl-tetrahydro-imidazo-(4,5-D)-imidazole-2,5-dione
Chemical formula	$C_8H_{14}N_4O_6$
Structural formula	
Molecular mass	262.23
CAS-No.	5395-50-6
EC-No.	226-408-0
Synonym/common name	tetramethylolacetylene diurea
Supplier	BASF
Chemical and physical properties of a 47% aqueous solution	
Appearance	clear, colourless or yellowish liquid
Content %	45–49 a.i. (HCHO content 20–24)
H ₂ O content %	48–52
pH at 20°C	6.5–7.5
Density g/ml (20°C)	1.21–1.22
Solubility	soluble in H ₂ O, ethanol and propylene glycol in all proportions
Stability	tetramethylolacetylene diurea results from the reversible addition of 4 mol HCHO to acetylene diurea (Glycoluril); the release of HCHO increases with pH and temperature
Toxicity data (source: BASF)	
LD ₅₀ oral	> 5000 mg/kg rat
In tests with rabbits not irritant to the skin, but irritant to eyes.	
Ecotoxicity:	
EC ₁₀ for bacteria (16 h)	> 100 mg/l
LC ₅₀ for <i>Leuciscus idus</i> (96 h)	> 100 mg/l

Antimicrobial effectiveness/applications

The tetramethylolacetylene diurea solution is an easy to handle formaldehyde delivery system which suits for the preservation of aqueous functional fluids. Together with other microbicides it can also be used for the formulation of disinfectants for hard surfaces, animal husbandry and sanitizers.

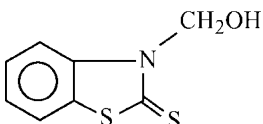
Table 47 Minimum inhibition concentrations (MIC) of tetramethylolacetylene diurea (47%) (Source: BASF)

Test organisms	MIC (mg/l)
<i>Staphylococcus aureus</i>	ATCC 6538 1000
<i>Escherichia coli</i>	ATCC 11229 1000
<i>Proteus mirabilis</i>	ATCC 14153 1000
<i>Pseudomonas aeruginosa</i>	ATCC 15442 1000
<i>Candida albicans</i>	ATCC 10231 5000

Microbicide group (substance class)	3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.4.11. Sodium N-hydroxymethyl-N-methyl-dithiocarbamate
Chemical formula	$C_3H_6NOS_2Na$
Structural formula	
Molecular mass	159.21
CAS-No.	60487-28-7
EC-No.	unknown
Chemical and physical properties of a 40% aqueous solution	
Appearance	yellow to brown coloured liquid
Content (%)	~40 (HCHO content ~7.5)
Density g/ml (25°C)	1.23
pH (0.1 g/l)	8–10
Stability	Releases formaldehyde; decomposes in acid media to CS ₂ , methylamine and HCHO; coloration with traces of heavy metals
Solubility	complete in H ₂ O and mixtures of alcohol and water
Toxicity data	
Moderately toxic by ingestion in single doses.	
Irritant to skin and eyes.	

Antimicrobial effectiveness/applications

According to its chemical composition the formulation exhibits antibacterial and antifungal activity. Concentrations of 2–10 mg/litre are used for slime control in pulp and paper mill systems. Pulp that may be held in storage for 8 h to 1 week may be protected by the addition of 0.01–0.03% of the 40% a.i. solution. The hydroxymethylated dithiocarbamate may also be used as a preservative for papermaking chemicals, such as glues, starch and clay slurries and coating formulations.

Microbicide group (substance class)	3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS
Chemical name	3.4.12. 1-Hydroxymethyl-2-thiono-1:2-dihydro-benzothiazol
Chemical formula	$C_8H_7NOS_2$
Structural formula	
Molecular mass	197.28
CAS-No.	3161-57-7
EC-No.	unknown
Synonym/common name	N-hydroxymethyl-benzothiazolin-2-thione, MBTT
Chemical and physical properties	
Appearance	yellow odourless crystals
Content	100 (HCHO content 15)
Melting point °C	130

Stability

the HCHO content is completely detectable by the Tanenbaum method; in alkaline solutions separation to HCHO and the corresponding salt of 2-mercapto-benzothiazole (MBT, 15.10.)

Solubility g/l (20°C)

180 in acetone, 220 in dioxane, 550 in dimethylformamide

1-Hydroxymethyl-2-thiono-1,2-dihydro-benzothiazol is listed under the reaction products of amides and formaldehyde because of its thiocarbonamide structure (Paulus, 1980).

Toxicity data: Not available.

Antimicrobial effectiveness/applications

As the formaldehyde contained in MBTT can be determined by quantitative analysis using the Tanenbaum method, it is not surprising that the range of activity of MBTT is such that gaps observed in the effective spectrum of MBT are successfully filled. The regularity of the activity spectrum of MBTT is demonstrated in Table 48; the efficacy of MBTT against *Pseudomonades* is 15-fold higher than that of MBT.

In alkaline functional fluids MBTT can serve as a broad spectrum preservative which additionally inhibits corrosion of non-ferrous metals as does MBT. Because of its solubility properties MBTT may be incorporated into lubricoolants.

Table 48 Minimum inhibition concentrations (MIC) of MBT and MBTT in nutrient agar

Test organism	MIC (mg/litre)	
	MBT	MBTT
<i>Aspergillus flavus</i>	500	500
<i>Aspergillus niger</i>	300	300
<i>Chaetomium globosum</i>	100	100
<i>Penicillium glaucum</i>	150	200
<i>Aureobasidium pullulans</i>	150	200
<i>Rhizopus nigricans</i>	500	500
<i>Bacillus mycoides</i>	500	150
<i>Bacillus punctatus</i>	450	100
<i>Bacillus subtilis</i>	700	200
<i>Bacterium vulgare</i>	500	100
<i>Escherichia coli</i>	200	150
<i>Pseudomonas aeruginosa</i>	8000	500
<i>Pseudomonas fluorescens</i>	8000	750
<i>Staphylococcus aureus</i>	450	150

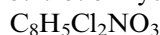
Microbicide group (substance class)

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

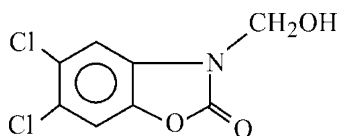
Chemical name

3.4.13. 3-Hydroxymethyl-5,6-dichloro-benzoxazolinone

Chemical formula



Structural formula



Molecular mass

234.04

CAS-No.

19986-60-8

EC-No.

unknown

Chemical and physical properties

Appearance

brownish, odourless powder

Content %

~100 (HCHO content 13)

Melting point °C

138–140

Stability	the fact that the HCHO content of the substance is detectable by the Tanenbaum method, characterizes it as a compound which separates formaldehyde under mild conditions; in NaOH solutions transformation to the corresponding sodium salt of 2-hydroxy-benzoxazol under elimination of HCHO
Solubility	practically insoluble in H ₂ O, soluble in polar solvents and NaOH solutions

Antimicrobial effectiveness

The starting product, the 5,6-dichlorobenzoxazolinone (11.7) contains the carbonamide or rather the carbamate structure and is a known fungicide especially for the protection of textile material. The introduction of the hydroxymethyl group leads to a compound with an effective spectrum more balanced than that of the starting product (Table 49).

Table 49 Minimum inhibition concentrations (MIC) of *N*-hydroxymethyl-5,6-dichlorobenzoxazolinone (A) and *N*-hydroxymethyl-5-chlorobenzoxazolinthione (B) (3.4.14.) in nutrient agar

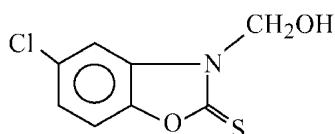
Test organism	MIC (mg/litre)	
	A	B
<i>Escherichia coli</i>	250	250
<i>Pseudomonas aeruginosa</i>	1500	800
<i>Aspergillus niger</i>	120	1500
<i>Penicillium glaucum</i>	60	500
<i>Rhizopus nigricans</i>	120	250

Microbicide group (substance class)

Chemical name
Chemical formula
Structural formula

3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

3.4.14. 3-Hydroxymethyl-5-chloro-benzoxazoline-2-thione
C₈H₆ClNO₂S



Molecular Mass
CAS-No.
EC-No.
Chemical and physical properties
Appearance
Content %
Melting point °C
Stability

215.66
3998-53-6
unknown

white, odourless needles
100 (HCHO content 14)

161

releases formaldehyde; the HCHO content is detectable by the Tanenbaum method; in NaOH solutions transformation to the water-soluble sodium salt of 5-chloro-2-mercapto-benzoxazol under elimination of HCHO

Solubility

practically insoluble in H₂O, soluble in polar solvents and alkaline solutions

Antimicrobial effectiveness (see Table 49.)

Up to now compounds A and B have not gained importance in practical applications as microbicides for the protection of materials, probably of not being cost effective.

Microbicide group (substance class)	3.5. REACTION PRODUCTS OF AMINO ACIDS WITH FORMALDEHYDE
Chemical name	3.5.1. Sodium 2-hydroxymethylaminoacetate
Chemical formula	$C_3H_6NO_3Na$
Structural formula	$HO-CH_2-NH-CH_2-COO^-Na^+$
Molecular mass	127.08
CAS-No.	70161-44-3
EC-No.	274-357-8
Registrations: Permitted for use in cosmetics in the EEC (No. 51) and the USA, not in Japan.	
Synonym/common name	sodium N-(hydroxymethyl)glycinate
Supplier	CLARIANT-NIPA, SUTTON LAB.

Chemical and physical properties of a 50% aqueous solution

Such a solution is prepared by reacting a mixture of glycine and sodium hydroxide in water with formaldehyde (2.1a). The addition of formaldehyde to the amino group of glycine leads to a molecule which may react further; it could dehydrate to a Schiff base, react with another molecule of glycine or polymerize (Berke & Rosen, 1982b). It is therefore more accurate to characterize the a.m. solution as an equilibrium mixture of compounds produced by the reaction of the sodium salt of glycine with formaldehyde.

Appearance	clear, colourless to pale yellow liquid with a mild pungent odour
Content %	50 (HCHO content approx. 12)
Boiling point/range °C (101 kPa)	99
Density g/ml (20°C)	1.28–1.30
pH value	10–12
Stability	instable at elevated temperatures, releases formaldehyde, increasingly with decreasing pH
Solubility (25°C)	highly soluble (> 50% w/w) in H ₂ O, propylene glycol, glycerine, < 0.1% in butylene glycol
Toxicity data	
LD ₅₀ Oral	Approx. 1200 mg/kg rat
dermal	> 2000 mg/kg rat

Bacterial mutagenicity tests were negative.

Moderately irritant on skin, mucous membranes and eyes.

No sensitization was observed in the guinea pig test.

Ecotoxicity data (source: CLARIANT-NIPA):

LC ₅₀ for Rainbow trout	93.8 mg a.i./l (96 h)
for Bluegill sunfish	> 100 mg a.i./l (96 h)
EC ₅₀ for Daphnia magna	46.5 mg a.i./l (48 h)

Antimicrobial effectiveness/applications

Sodium hydroxymethylglycinate is a formaldehyde releasing compound; its formaldehyde content is detectable with the Tanenbaum method. The antimicrobial activity corresponds to its formaldehyde content. The compound inhibits preferably the growth of bacteria, but at higher concentrations also the growth of fungi and yeasts. High water solubility accompanied by a favourable partition coefficient make sodium hydroxymethylglycinate an appropriate microbicide for the in-can/in-tank protection of aqueous functional fluids. It is mainly recommended for the preservation of cosmetics, especially hair care products, to meet a current need for a safe but economical microbicide. Typical use concentrations range from 0.1–1% (50% sodium hydroxy-methylglycinate solution). The product should not be exposed to temperatures in excess of 60°C for prolonged periods; in hot processes it should be added during the cooling stage at the lowest practical temperature.

In the EC list of preservatives permitted for use in cosmetic formulations sodium hydroxymethylglycinate is registered with a max. concentration of 0.5% a.i.

Table 50 Minimum inhibition concentrations (MIC) of sodium hydroxymethylglycinate (50%) (Source: CLARIANT-NIPA)

Test organisms	MIC in %
<i>Pseudomonas aeruginosa</i>	0.125
<i>Escherichia coli</i>	0.125
<i>Proteus vulgaris</i>	0.10
<i>Salmonella enteritidis</i>	0.10
<i>Staphylococcus aureus</i>	0.125
<i>Micrococcus luteus</i>	0.125
<i>Candida albicans</i>	0.80
<i>Saccharomyces cerevisiae</i>	0.10
<i>Aspergillus niger</i>	0.275
<i>Penicillium purpurogenum</i>	0.10

Microbicide group (substance class)

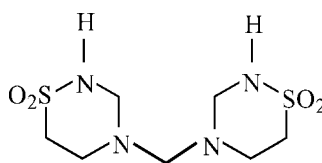
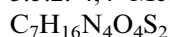
Chemical name

Chemical formula

Structural formula

3.5. REACTION PRODUCTS OF AMINO ACIDS WITH FORMALDEHYDE

3.5.2. 4,4'-Methylenebis-(1,2,4-thiadiazine-1,1-dioxide)



Molecular mass

CAS-No.

EC-No.

Synonym/common name

284.36

19388-87-5

243-016-5

bis(1,1-dioxo-perhydro-1,2,4-thiadiazin-4-yl)methane,
Taurolidine

Chemical and physical properties

Content (%)

Stability

Solubility

100 (HCHO content 32)

releases formaldehyde in aqueous media

very soluble in H₂O, and polar organic solvents*Antimicrobial effectiveness/application*

The starting material for the synthesis of Taurolidine is 2-aminoethane-sulphonic acid (Taurine) which by the addition of 2 M NH₃ and 3 M CH₂O in a condensation reaction is converted to Taurolidine. The spectrum of effectiveness corresponds to that of formaldehyde, however, according to findings of Myers *et al.* (1980) and Allwood & Myers (1981) the activity of Taurolidine is considerably greater than that of formaldehyde; apparently the 4-hydroxymethyl-1, 1-dioxoperhydro-1,2,4-thiadiazine which is released in the first step on hydrolysis of Taurolidine plays an important role in the mechanism of Taurolidine's antimicrobial activity.

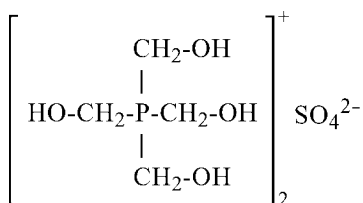
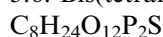
Taurolidine is known as a bactericidal chemo-therapeuticum. An extensive report about Taurolidine-bacteriology in vitro has been published by Brodhage & Pfirrmann (1985). However, as preservative for water-based functional fluids Taurolidine has not got importance.

Microbicide group (substance class)

Chemical formula

Struntural formula

3.6. Bis(tetrakis(hydroxymethyl)phosphonium) sulfate



Molecular mass	406.29
CAS-No.	55566-30-8
EC-No.	259-709-0
EPA-Reg.TSCATS	Data base, January 2001
Synonym/common name	octakis(hydroxymethyl)phosphoniumsulfate, THPS
Supplier	SIGMA-ALDRICH, TENNECO-ALBRIGHT & WILSON

Chemical and physical properties of a
70% aqueous solution

Appearance	clear, straw coloured fluid of pungent odour
Content(%)	70±1 (HCHO content ~30)
Density g/ml (25°C)	1.36
pH (25°C)	approx. 5
Stability	releases formaldehyde; the HCHO content is detectable by the Tanenbaum method; sensitive to alkalis and oxidizing agents: formation of tris(hydroxymethyl)phosphine and/or tris(hydroxymethyl)phosphine oxide
Solubility	complete in water

Toxicity data (source: Tenneco/Sigma-Aldrich)

LD ₅₀ oral	248 mg/kg rat

Strongly irritant to the skin, eyes and mucous membranes.

Not mutagenic in bacterial test systems. No evidence of carcinogenicity after a two-year gavage study in rats and mice.

Ecotoxicity:

LC ₅₀ for Rainbow trout	99 mg/l (96 h)
THPS is inherently biodegradable.	

Antimicrobial effectiveness/applications

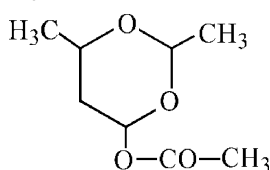
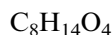
THPS is a quaternary phosphonium salt which releases formaldehyde. However, the tris(hydroxymethyl)phosphine which forms in alkaline media, exhibits a special antimicrobial activity. It is able to reduce disulphide amino acid (e.g. cystine) residues of microbial cell entities to –SH amino (e.g. cysteine) components; the phosphine is converted to phosphine oxide. See also Part I, chapter 5.4. THPS is claimed to be effective against algae, fungi and bacteria and recommended for use in badly fouled cooling systems and injection water for secondary oil recovery as a slimicide which is especially active against sulphate reducing bacteria at addition rates of 50–500 mg/litre. THPS may also be used as a preservative for the protection of water-based functional fluids. According to a report of Suzuki (1999) a synergistic slime control effect is achieved, if a combination of THPS and o-phthalaldehyde (2.11.) is introduced to white water.

Table 51 Minimum inhibition concentrations (MIC) of the THPS formulation in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	400
<i>Aeromonas punctatum</i>	400
<i>Bacillus mycoides</i>	400
<i>Bacillus subtilis</i>	400
<i>Escherichia coli</i>	800
<i>Leuconostoc mesenteriodes</i>	400
<i>Proteus mirabilis</i>	800
<i>Pseudomonas aeruginosa</i>	≥800
<i>Pseudomonas fluorescens</i>	≥800
<i>Staphylococcus aureus</i>	50
<i>Candida albicans</i>	> 800
<i>Candida krusei</i>	600
<i>Rhodotorula mucilaginosa</i>	400
<i>Torula rubra</i>	> 800
<i>Torula utilis</i>	800
<i>Aspergillus niger</i>	> 800
<i>Chaetomium globosum</i>	600
<i>Penicillium brevicaulis</i>	> 800

4 Acetaldehyde releasing compound

Microbicide group (substance class)
 Chemical name
 Chemical formula
 Structural formula

4. ACETALDEHYDE RELEASING COMPOUND**4.1. 6-Acetoxy-2,4-dimethyl-1,3-dioxane**

Molecular mass

174.18

CAS-No.

828-00-2

EC-No.

212-579-9

EPA-Reg.

for use in a great variety of waterbased industrial functional fluids

FDA

clearances for adhesives

Synonym/common name

2,6-dimethyl-1,3-dioxane-4-yl-acetate, Dimethoxane,
 Bioban DXN

Supplier

DOW-ANGUS

Chemical and physical properties

Appearance

colourless to yellow fluid with a typical odour

Content (%)

93

Acetaldehyde content

3 Mol (77%)

Boiling point/range °C (0.4 kPa)

66–68

Solidification point °C

< 25

Density g/ml (25°C)

1.055–1.070

Vapour pressure hPa (23°C)

0.15

Refractive index nD (20°C)

1.4270–1.4320

Flash point °C

> 61

pH at 25°C

approx. 5.8

Stability

tolerates heat for a short while; sensitive to acids, because of its acetal nature; hydrolyses slowly in aqueous solutions to acetaldehyde (2.2) and acetic acid (7.1.2.); in the presence of ammonia and/or amines yellowing and inactivation occurs; the compound is not active in formulations with a pH over 9

Solubility

miscible with water; very soluble in organic solvents and oils

Toxicity data

LD₅₀ oral

2.457 mg/kg rat

dermal

> 2.000 mg/kg rat

inhalative

> 4 mg/l for rats

In tests with rabbits not irritant to the skin, but to mucous membranes. – Sensitization may occur.

Not mutagenic.

90-day dermal toxicity in rats: NOEL: 100 mg/kg/day.

Ecotoxicity:

LC₅₀ for rainbow trout

> 37 mg/l (96 h)

daphnia magna

> 24 mg/l (48 h)

Antimicrobial effectiveness/application

Dimethoxane is effective against bacteria, yeasts and fungi (see Table 52). As a preservative for functional fluids, such as detergent solutions, emulsions, water based paints, metal working fluids Dimethoxane profits from its insensitiveness to non-ionic, anionic and cationic emulsifiers and detergents. Addition rates: 0.1–0.2%. Most

effective is Dimethoxane in formulations with pH values between 3 and 8; limited activity exhibits the compound at pH values between 8 and 9. Dimethoxane in aqueous formulations liberates acetic acid, so that pH drop may occur; for pH adjustment one can add sodium carbonate (not NaOH or KOH).

Table 52 Minimum inhibition concentrations (MIC) of dimethoxane
(Source: DOW-ANGUS)

Test organism	MIC (mg/l)
Bacteria	
<i>Escherichia coli</i>	625
<i>Enterobacter aerogenes</i>	625
<i>Pseudomonas aeruginosa</i>	625
<i>Pseudomonas fluorescens</i>	625
<i>Salmonella choleraesuis</i>	312
<i>Salmonella typhosa</i>	625
<i>Shigella sonnei</i>	625
<i>Bacillus subtilis</i>	625
<i>Brevibacterium ammoniagenes</i>	625
<i>Staphylococcus aureus</i>	1250
Fungi	
<i>Aspergillus flavus</i>	1250
<i>Aspergillus niger</i>	1250
<i>Aspergillus oryzae</i>	1250
<i>Aspergillus terreus</i>	1250
<i>Penicillium piscarium</i>	625
<i>Penicillium sp.</i>	1250
<i>Candida albicans</i>	1250
<i>Pityrosporum ovale</i>	625
<i>Saccharomyces cerevisiae</i>	2500

5 Succinaldehyde releasing compound

Microbicide group (substance class)

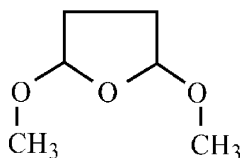
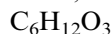
Chemical name

Chemical formula

Structural formula

5. SUCCINALDEHYDE RELEASING COMPOUND

5.1. 2,5-Dimethoxytetrahydrofuran



Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

132.16

696-59-3

211-797-1

2,5-dimethoxyoxolane, succindialdehydedimethylacetal

BASF, SIGMA-ALDRICH

Chemical and physical properties

Appearance

Content %

Boiling point °C (101 kPa)

Density g/ml (20° C)

Refractive index nD (20° C)

Flash point °C

pH (30% solution in H₂O)

Stability

clear, colourless liquid

~99

145–147

1.020

1.4180

35

~5

sensitive to air, moisture, light and heat; storage at temperatures higher than 40°C result in discolouration of the product; hydrolyzes in acidic media, as it is characteristic for acetals, to succinaldehyde (2.4.)

Solubility

soluble in water up to approx. 10%; completely soluble in a range of polar solvents such as methanol, ethanol and propanol

Toxicity data (source: BASF)

LD₅₀ oral > 2000 mg/kg rat
 dermal > 4000 mg/kg rabbit

Irritant to mucous membranes.

Ecotoxicity:

LC₅₀ for *Leuciscus idus* > 100 mg/l (96 h)

Antimicrobial effectiveness/applications

2,5-Dimethoxytetrahydrofuran does not exhibit antimicrobial activity as long as it is not in a neutral to acid medium favouring its hydrolysis, which leads to the liberation of succinaldehyde.

Succinaldehyde is a microbicide which disposes of a broad spectrum of effectiveness covering Gram-positive and Gram-negative bacteria (including bacterial spores), yeast, fungi and certain viruses. It has a rapid speed of kill, even in the presence of protein, making it to an ideal active ingredient for use in disinfectants, e.g. for surgical instruments.

The MIC listed in Table 53 demonstrate the microbistatic activity of succinaldehyde.

Table 53 Minimum inhibition concentrations (MIC) of succinaldehyde
 (Source: BASF Specialty Chemicals)

Test organism	MIC (mg/l)
<i>Staphylococcus aureus</i> ATCC 6538	2500
<i>Escherichia coli</i> ATCC 11229	2500
<i>Proteus mirabilis</i> ATCC 14153	2500
<i>Pseudomonas aeruginosa</i> ATCC 15442	2500
<i>Candida albicans</i> ATCC 10231	5000

6 2-Propenal-releasing-compound

Microbicide group (substance class)

Chemical name

6. 2-PROPENAL-RELEASING-COMPOUND

6.1. Copolymer, base: 2-propenal (2.6.) and propane-1,2-diol (1.14.)

Molecular mass

4.000

CAS-No.

191546-07-3

EC-Notification-No.

329

Synonym/common name

copolymer, base: acrolein and propylene glycol, APC

Supplier

DEGUSSA-CREANOVA-COLORENTREND

Chemical and physical properties

Appearance

light yellow liquid with a characteristic odour

Content (%)

50 (polymerised 2-propenal; weight ratio 2-propenal: propane-1,2-diol approx. 1:1)

Boiling point/range °C (101 kPa)

starting at ~100

Density g/ml (20°C)

1.10–1.13

Vapour pressure hPa (20°C)

13

Viscosity mPas (20°C)

~600

Stability

stable under normal conditions; primary and secondary amines reduce efficiency

Solubility

soluble/dispersible in water and alcohols; poor solubility in oil and hydrocarbons

Toxicity data (source: DEGUSSA)

LD₅₀ oral > 2.150 mg/kg rat
 > 2.000 mg/kg rabbit

Irritant to skin and mucous membranes. Strong irritation on the eye. Sensitization possible through skin contact.

Ecotoxicity:

LC ₅₀ for <i>Leuciscus idus melanotus</i>	14 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	28 mg/l (48 h)
EC ₅₀ for <i>Pseudomonas putida</i>	269 mg/l (16 h)

Antimicrobial effectiveness/applications

Under conditions of application APC releases acrolein only slowly; accordingly it does not exhibit short-term efficiency. However, APC is suitable for the purpose of preservation (long-term efficiency) of a great variety of water-based functional fluids, e.g. aqueous dispersions, paints, polymers, amine-free metalworking fluids, cooling water circuits. Addition rates: 0.05–0.3%. One has to keep in mind that for the in-can protection considerably smaller doses than suggested by MIC values are required.

7 Phenolics

Phenol, also termed carboic acid (7.1.), and the phenol derivatives could also have been discussed under '8. ACIDS', because of their acidity and the resulting capacity to form stable salts. However, since they represent a large, important substance class, they are described in a separate section.

Coal tars, which form during the destructive distillation of coal, are one source of phenol and phenol derivatives. Today, however, the production of microbicidally effective phenol derivatives for the protection of materials and the formulation of disinfectants from coal tars has for the most part been replaced by efficient, industrial-scale, target-specific syntheses which supply very pure active substances. The so-called pressure phenol synthesis which uses chlorobenzene produces phenol as well as 2-phenylphenol (7.4.1.) and 4-phenylphenol (7.4.2) as by-products. Today a more significant synthesis for the production of 2-phenylphenol is one starting with cyclohexane via cyclohexanone. This is first condensed to 2-cyclohexenylcyclohexanone and its catalytic dehydration provides a good yield of very pure 2-phenylphenol. On heating benzyl chloride in excess phenol, 2- and 4-benzylphenol (7.2.6.) form. These in turn can be used to produce the corresponding chloro-benzylphenols by adding SO₂Cl₂, e.g. 2-benzyl-4-chlorophenol = Chlorophen (7.3.5.). The most effective compounds form on chlorination of phenol and phenol derivatives. On the other hand, chlorophenols are the starting products for the manufacture of the microbicides Dichlorophen (7.7.3.) and Hexachlorophen (7.7.4.) which form during irreversible reaction of formaldehyde with 4-chlorophenol (7.5.1.) or 2,4,5-trichlorophenol (7.5.3.).

However, the development of microbicidally active phenol derivatives started from phenol itself, from carboic acid, the antiseptic properties of which were detected in 1860 and first used by Lister in 1867 to kill bacteria on medical instruments, surgical dressings and wounds. The development of chemical disinfection revolutionized progress in hospitals, particularly in surgery. It was possible to successfully employ and develop surgical techniques which in the past had been feasible but impractical because of the associated unavoidable massive and generally fatal infections.

The substance class of microbicidally active phenol derivatives, in short phenolics, was also developed. Hundreds of different derivatives were isolated, synthesized and investigated with the aim of finding phenol derivatives which were more effective and at the same time less toxic and less irritating to the skin than the parent chemical carboic acid. Another objective was to find microbicidal phenol derivatives with chemico-physical properties which make them suitable for the protection of materials. This development, which can now be considered as completed, provided knowledge on the relationship between structure and effectiveness and on the mechanism of action of phenol derivatives. This is summarized in the following.

The free hydroxyl group together with the aromatic system of the phenyl ring constitutes the reactive centre of the phenol molecule. Its reactivity can be influenced in various ways by introducing different substituents into the phenyl nucleus.

Alkylated phenols (7.2.) are less soluble in water and less acidic than phenol. Furthermore, the acidity decreases in the direction m → p → o-substitution. Nevertheless, the alkyl phenols which are of interest as microbicides are still capable of forming alkaline salts which dissolve easily in water. With reduced water-solubility, a property which is important for the use of phenol derivatives as microbicides, also changes the ratio of the distribution between the aqueous and non-aqueous phases, including bacterial phases. However, not only the distribution factor but also the capability of reducing surface tension changes; in fact it changes increasingly with the length of the alkyl chain. As to be expected for membrane-active substances, a consequence of these property changes is increasing antimicrobial effectiveness with increasing alkyl chain length. This reaches a maximum with the unbranched C₆ chain in the p-position. Branched chains with the same number of C atoms do not increase the effectiveness of the corresponding phenol derivatives to such an extent. The introduction of alkyl chains with more than 6 C atoms does not lead to a further rise in effectiveness. This is due to the decreasing water-solubility.

Halogenation of phenol also leads to phenol derivatives which are much more effective than the starting substance. At the same time, the dissociation constant increases with an increase in the number of halogen atoms, i.e. the acidic character of the phenol derivatives becomes more distinctive. The combination of alkylation and halogenation (the latter with preference in the p-position) has led to microbicides which have attained great practical significance as substances for the protection of materials and disinfection, e.g. p-chloro-o-benzylphenol (7.3.5.), p-chloro-m-cresol (7.3.1.), p-chloro-m-xylenol (7.3.2.).

For the sake of completeness, the nitration of phenol and cresol must be mentioned. It particularly strengthens the bactericidal effect. Furthermore, nitrophenols have specific biological properties since they are able to interfere with oxidative phosphorylation. However, nitrophenols (7.8.) are no longer of practical importance as microbicides.

As already mentioned, phenol derivatives are membrane-active microbicides. They adsorptively coat the surface of the microbe cell, then, at a higher concentration, they are dissolved more or less rapidly and well by lipoids depending on their chemico-physical properties (see above). They attack the cell wall and penetrate into the cell. There are reactions with the protoplasm and the cellular protein; enzymes are also inhibited as a result; oxidoreductases and the enzymes of carbohydrate and protein metabolism react particularly sensitively. Whether the phenol derivatives act microbistatically or microbicidally is purely a question of the application concentration. At low concentrations in ambient medium, there is only reversible adsorption of the phenolic active substance at the cytoplasmic membrane and the related inhibiting effect. As stated above the cell wall is only penetrated and destroyed and the microbe cell killed at higher concentrations. The penetration capability of phenolic compounds depends on their hydrophobicity which one quantifies by the octanol/water partition coefficient P , respectively $\log POW$. Phenolics exhibit optimal antimicrobial activity in their undissociated state, that means in acid to neutral media, only. In this respect the pK_a values of phenol derivatives are of interest; they indicate the pH value at which 50% of the corresponding phenol is present in its undissociated state. Thus the hydrophobicity of phenol derivatives and acids (8.) is pH dependent. Therefore it makes sense, to quantify the hydrophobicity of phenolic compounds by the ionization-corrected hydrophobicity descriptor D . Values of $\log D$ were calculated by Schultz et al. (1997) and Ren (2002) according to the relationship between D and P (the octanol/water partition coefficient at pH 7.35):

$$P = D(1 + 10^{pH - pK_a})$$

pK_a and $\log DOW$ values for some important phenolic microbicides are listed in Table 54.

Table 54 pK_a and $\log DOW$ values of selected phenolic microbicides

Phenol derivatives	pK_a	$\log DOW$
2-Phenylphenol	11.6	3.090
Benzylphenol	11.6	
3-Methyl-6-isopropylphenol (Thymol)	10.6	
2-Benzyl-4-chlorophenol	9.7	
4-Chloro-3,5-dimethylphenol (PCMX)	9.7	3.483
4-Chloro-3-methylphenol (PCMC)	9.6	2.984
5,5'-Dichloro-2,2'-dihydroxydiphenylmethane	8.7/12.6	
2,4,6-Trichlorophenol	8.5	2.326
Methyl-4-hydroxybenzoate	8.5	1.985
Salicylic acid	3.0	-2.183

For practical use of microbicidal phenol derivatives, it is very frequently necessary to improve their solubility, especially their solubility in water and hence to shift the distribution ration towards the aqueous phase. If for this purpose phenol derivatives are converted into sodium or potassium salts which dissolve easily in water, it is important to remember that the dissociated phenolate anion is not nearly as effective as the undissociated phenol. As solubilization is an urgent requirement for effectiveness, one has to find a compromise when using alkalis or amines as solubilizing agents. Sometimes half of the alkali quantity required for salt formation is sufficient to attain adequate solubility in water; in other cases it is necessary to use excess alkali. It is particularly advantageous if one has and can use phenol derivatives which show considerable water-solubility in their undissociated condition, however this is seldom the case. One example is p-chloro-m-cresol with a water-solubility of 0.4%. For many applications, alkaline phenolate solutions are used as transport media to deposit the active ingredients in the form of sparingly soluble and non-leachable precipitates on acidic surfaces such as wood, leather, cardboard or textiles. Frequently the influence of the carbon dioxide in the atmosphere is enough to release and fix the free phenol on material impregnated with phenolate solution. In aqueous stock-piled phenolate solutions with slight excess alkalinity, the introduction of CO_2 and the related reduction of the pH value can cause precipitation of the

free ingredients. Since such precipitations are undesirable and restrict applicability of the phenolate solutions, counteractions must be taken: storage in closed reservoirs or provision of distinct excess alkalinity.

Corresponding to the mechanism of action, the microbicidal phenol derivatives are effective over a wide spectrum including bacteria, yeasts and fungi. However, there are different effectiveness peaks depending on the type and number of the substituents. Therefore the combined application of various phenol derivatives sometimes allows the application concentrations to be reduced; this is to be aimed at for many reasons. Phenol derivatives are not effective against resistant bacterial spores, at least not at room temperature. However, they are very effective against lipophilic viruses but deficient in their activity against viruses with hydrophilic properties.

Table 55 presents an overview of the activity spectra of phenol derivatives which have gained especial importance as microbicides.

Table 55 Minimum inhibition concentrations (MIC) of phenol derivatives in nutrient agar (for abbreviations see Figure 15)

Test organism	MIC (mg/litre)						
	OPP	BP	CBP	PCMC	PCMX	DC	PCP
<i>Aeromonas punctata</i>	200	100	10	200	100	50	10
<i>Bacillus subtilis</i>	100	100	10	150	75	100	10
<i>Escherichia coli</i>	200	500	3500	250	200	100	500
<i>Leuconostoc mesenteroides</i>	100	100	10	200	100	5	35
<i>Proteus vulgaris</i>	200	200	100	200	200	50	100
<i>Pseudomonas aeruginosa</i>	1500	5000	5000	800	1000	> 5000	500
<i>Pseudomonas fluorescens</i>	1500	5000	> 5000	800	500	3500	500
<i>Staphylococcus aureus</i>	100	100	20	200	100	5	10
<i>Desulfovibrio desulfuricans</i>	50	100	50	35	50	20	35
Formaldehyde resistant bacteria	750	4000	> 5000	250	300	300	250
<i>Candida albicans</i>	100	100	50	200	75	50	35
<i>Torula rubra</i>	100	100	50	50	100	50	100
<i>Alternaria tenuis</i>	100	75	20	200	75	50	1
<i>Aspergillus flavus</i>	85	200	75	100	100	50	100
<i>Aspergillus niger</i>	75	100	100	100	100	100	50
<i>Aureobasidium pullulans</i>	35	100	20	30	50	35	20
<i>Chaetomium globosum</i>	60	50	20	80	50	20	20
<i>Cladosporium herbarum</i>	60	200	100	200	100	200	50
<i>Coniophora puteana</i>	50	35	5	100	35	2	35
<i>Lentinus tigrinus</i>	100	75	20	3500	75	5	10
<i>Paecilomyces variotii</i>	100	100	50	200	100	50	50
<i>Penicillium citrinum</i>	35	100	75	100	50	50	50
<i>Penicillium glaucum</i>	80	100	50	100	35	50	50
<i>Polyporus versicolor</i>	65	100	50	5000	75	50	20
<i>Rhizopus nigricans</i>	50	100	50	100	100	35	15
<i>Sclerophoma pityophila</i>	100	100	20	100	75	20	10
<i>Stachybotrys atra corda</i>	50	35	20	100	35	15	15
<i>Trichoderma viride</i>	75	200	100	140	100	50	200
<i>Trichophyton pedis</i>	20	20	10	100	50	10	10

In detergent solutions, primarily in solutions of non-ionic detergents but also in solutions of anionic detergents, phenol derivatives frequently only display the antimicrobial effect expected of them at unusually high concentrations. This also applies to the esters of p-hydroxy-benzoic acid (8.1.11.) which can also be considered as phenol derivatives. The cause is inclusion of the phenolic ingredients in the micelles which detergents form as soon as their concentration exceeds the critical micellization concentration (see 18.). Below this concentration which is characteristic for different detergents, the micelles dissolve again, releasing the active phenol derivatives.

It is frequently claimed that phenolics, particularly chlorinated phenolics, possess high oral toxicity, are percutaneously toxic and are generally difficult to degrade. Such blanket condemnations of the phenolics are based on invalid generalizations not confirmed by the facts. It is therefore in no way justified to disqualify the whole class of microbicidal phenolic compounds out of hand simply because some of them are described, correctly, as highly toxic, persistent, ecotoxic or dioxin-containing. The phenol derivatives enumerated in the following have many applications as microbicides, not only because they have broad spectra of effectiveness or because of the special nature of their chemical and physical properties, but also because they include derivatives whose toxicological properties and effects on ecosystems comply with the demands which should be made on microbicides for material protection by a civilization with an awakened sense of responsibility for the environment. There is nothing inappropriate or irresponsible in the continued large-scale use of those phenol derivatives as microbicides for the protection of materials and as active ingredients for disinfectants. There would appear to be little point in listing the phenols in order of preference according to toxicity data, since the relevance of these data depends on the intended application. Thus o-phenylphenol will continue to be the only phenol derivative which is used

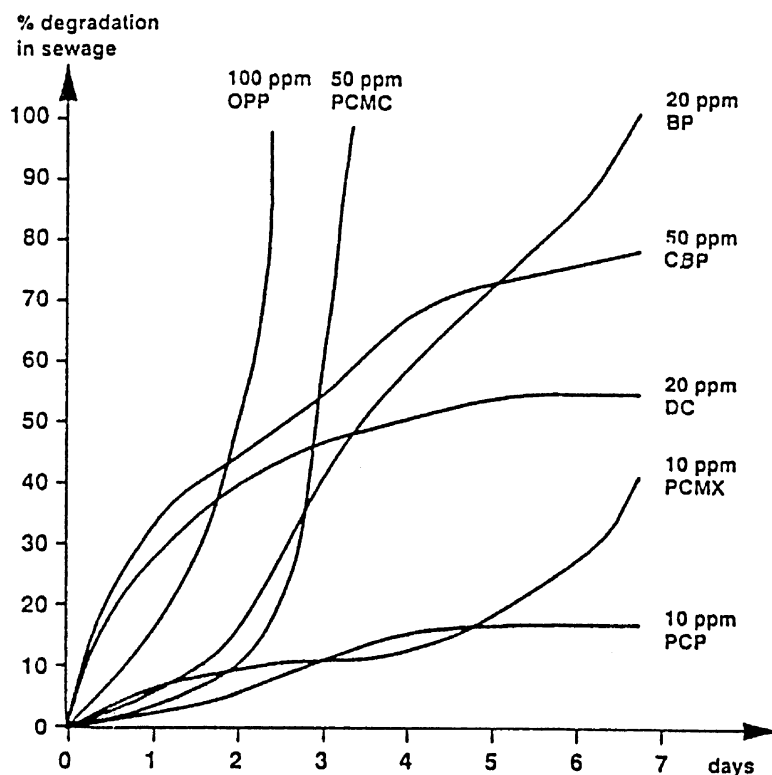


Figure 15 Decrease in concentration of phenolics in activated sludge (Paulus & Genth, 1983). (OPP, *o*-phenyl-phenol; PCMC, *p*-chloro-*m*-cresol; BP, benzyl-phenol; CBP, *p*-chloro-*o*-benzyl-phenol; DC, (2,2'-dihydroxy-5,5'-dichloro)-diphenyl methane; PCMX, *p*-chloro-*m*-xyleneol; PCP, pentachlorophenol.)

to preserve citrus fruits because it has the most favourable toxicity data, which is particularly important in this application. It should be pointed out here that there is no foundation for allegations to the effect that phenolics are incapable of undergoing biodegradation. As can be seen from the curves in Figure 15 there are phenolics which are degraded easily and quickly, whereas others, e.g. pentachlorophenol, are degraded slowly and only at low concentrations. But microbial degradation of phenolics does not occur in biological sewage treatment plants only; also the aquatic environment possess a considerable biodegradation potential against phenolics, even against pentachlorophenol (Liu, 1989).

Mendoza-Cantú et al. (2000) have found that the white rot fungus *Phanerochaete chrysosporium*, which is recognized for its ability to metabolize PCP, reduces PCP levels in contaminated soil in a short period of time without generation of toxic compounds; it is proposed to use *P. chrysosporium* in soil bioremediation.

From the facts there is no foundation for a blanket rejection of phenol derivatives. On the contrary it must be assumed, for many reasons, that it will not be possible to dispense with special phenolics as microbicides for material protection. After all, some of them, such as *o*-phenylphenol and *p*-chloro-*m*-cresol, meet the requirement now expected of such microbicides very satisfactorily.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

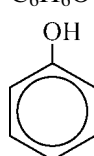
EPA – TSCATS

Synonym/common name

7. PHENOLICS

7.1. Phenol

C_6H_6O



94.11

108-95-2

203-632-7

Data Base, Jan. 2001

Carbolic acid, monohydroxybenzene

Chemical and physical properties

Appearance	colourless needles with a strong characteristic odour; reddish discolouration, if exposed to air
Content (%)	~100
Boiling point/range °C (101 kPa)	182
Melting point °C	41–43
Density g/ml (20°C)	1.071
Vapour pressure hPa (20°C)	6.19
Flash point °C	79
Upper flammability limit % v/v i. air	8.6
Lower flammability limit % v/v i. air	1.7
Dissociation constant	1.05×10^{-10}
Stability	volatile with water vapour; the electron affinity of the aromatic nucleus polarizes the hydroxy group (dissociation of hydrogen ions) and creates the acid character of phenol
Solubility g/l (16°C)	~67 in H ₂ O; highly soluble in alcohols, ether, chloroform, moderately soluble in benzene and alkaline solutions
Toxicity data	
LD ₅₀ oral	317 mg/kg rat
dermal	669 mg/kg rat
intraperitoneal	127 mg/kg rat
subcutaneous	460 mg/kg rat
LC ₅₀ inhalative	316 mg/m ³ for rats
LD ₅₀ intravenous	112 mg/kg mouse
Phenol is corrosive for the skin, eyes and mucous membranes and exhibits its toxic effect also percutaneously. 500 mg/24 h cause severe irritation on the rabbit skin; 5 mg only, create severe eye irritation.	
Exposure limits (occupational)	France/Germany 19(5) mg/m ³ (ppm) UK 20(5) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

The minimum inhibition concentrations of phenol for bacteria, yeast and fungi are significantly higher (> 1.000 mg/l in nutrient agar) than those of other phenol derivatives. Moreover phenol is highly toxic. Therefore it is not any longer important as a preservative or as an active ingredient in disinfectants.

Microbicide group (substance class)

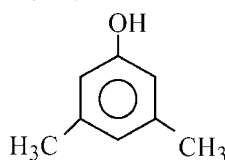
Chemical name

Chemical formula

Structural formula

7.2. ALKYLPHENOLS

7.2.1. 3,5-Dimethylphenol

C₈H₁₀O

Molecular mass

CAS-No.

EC-No.

Synonym/common name

122.17

108-68-9

604-037-00-9

Xylenol

Chemical and physical properties

Appearance	white to yellow crystals with phenolic odour
Content (%)	~100
Boiling point/range °C (101 kPa)	222
Melting point °C	65–66
Vapour pressure hPa (25°C)	0.05

Flash point °C	80
Log POW	2.35
Stability	volatile with water vapour
Solubility g/l (25°C)	5 in H ₂ O; very soluble in alcohols, acetone, ether and other organic solvents

Toxicity data

More toxic and corrosive to skin and mucous membranes than cresols (monomethylphenols).

Exposure limits (occupational) Germany 19(5) mg/m³ (ppm)

Ecotoxicity: The substance is harmful to aquatic organisms.

Antimicrobial effectiveness/applications

Xylenol may be used as an active ingredient in preservatives and disinfectants, but is, however, no longer of practical importance in these applications.

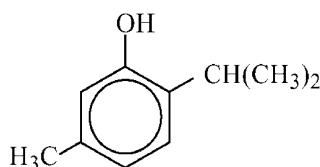
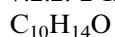
Table 56 Minimum inhibition concentrations (MIC) of xylenol in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	500
<i>Staphylococcus aureus</i>	1000
<i>Aspergillus niger</i>	500
<i>Chaetomium globosum</i>	500
<i>Penicillium glaucum</i>	500

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.2. ALKYLPHENOLICS

7.2.2. 2-Isopropyl-5-methylphenol



Molecular mass

150.22

CAS-No.

89-83-8

EC-No.

201-944-8

EPA-Reg. –TSCATS

Data Base, Jan. 2001

Synonym/common name

Thymol, 1-hydroxy-2-isopropyl-5-methylbenzene, 5-methyl-2-(1-methylethyl) phenol

Supplier

HAARMANN & REIMER CORP., SIGMA-ALDRICH

Chemical and physical properties

Appearance

colourless translucent crystals with an odour similar to thyme

Content (%)

~100

Boiling point/range °C (101 kPa)

232

Melting point °C

49-51

Density g/ml

0.965

Vapour pressure hPa (64°C)

1.33

Refractive index n_D (20°C)

1.5227

Flash point °C

102.22

pK_a value

10.62

pH (~0.1% in H₂O)

~6

Log POW

3.3

Stability	volatile with water vapour; Thymol is found in nature, e.g. in plants, such as thyme; it is manufactured synthetically, e.g. ex m-cresol
Solubility g/l(20°C)	1.0 in H ₂ O; highly soluble in alcohols, ether, chloroform, fatty oils and alkaline solutions
Toxicity data	
LD ₅₀ oral	980 mg/kg rat 640 mg/kg mouse
LD ₅₀ intraperitoneal	110 mg/kg mouse
subcutaneous	243 mg/kg mouse
intravenous	100 mg/kg mouse

Irritant to skin, eyes and mucous membranes.

In comparison to phenol (7.1.) Thymol is of lower toxicity and also not as corrosive as phenol. One can assume that the reason for that is the poorer water solubility of Thymol.

Ecotoxicity: EC₅₀ for activated sludge organisms 40 mg/l (method: OECD 209)

Antimicrobial effectiveness/applications

The broad spectrum of effectiveness and the high antimicrobial activity of iso-propyl-methylphenols is enlightened by the MIC in Table 57. In spite of that the isopropyl-methylphenols have not gained much importance as preservatives for material protection or as active ingredients in disinfectants. The reason for this is the distinctive odour of these phenol derivatives, their poor water solubility and their unfavourable distribution between water and organic phases. Last but not least they are more expensive than other phenol derivatives with more favourable properties. Worthy of note are antioxidant actions of the isopropyl-methylphenols (Aeschbach et al., 1994).

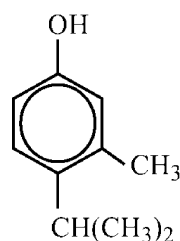
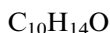
Table 57 Minimum inhibition concentrations (MIC) of isopropyl-methylphenols in nutrient agar

Test organism	MIC (mg/litre)		
	Thymol	o-Cymenol	Carvacrol
<i>Bacillus subtilis</i>	200	200	
<i>Escherichia coli</i>	500	750	200
<i>Pseudomonas aeruginosa</i>	1000	1000	
<i>Pseudomonas fluorescens</i>	1000	300	
<i>Staphylococcus aureus</i>	200	200	200
<i>Aspergillus niger</i>	200	200	200
<i>Alternaria tenuis</i>	200	200	
<i>Aureobasidium pullulans</i>	200	200	
<i>Chaetomium globosum</i>	100	100	100
<i>Coniophora puteana</i>	50	50	
<i>Lentinus tigrinus</i>	150	100	
<i>Penicillium glaucum</i>	350	500	200
<i>Polyporus versicolor</i>	200	200	
<i>Sclerophoma pityophila</i>	200	500	
<i>Trichoderma viride</i>	350	500	

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.2. ALKYLPHENOLS

7.2.3. 4-Isopropyl-3-methylphenol



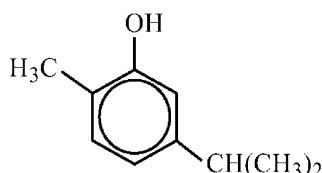
Molecular mass	150.22
CAS-No.	3228-20-2
EEC-No.	38
Synonym/common name	o-Cymen-5-ol, 4-isopropyl-m-cresol, 1-hydroxy-4-isopropyl-3-methylbenzene
Supplier	NAARDEN, OSAKA KASEI
Chemical and physical properties	
Appearance	colourless crystals with a characteristic, distinctive odour
Content (%)	~100
Boiling point/range °C (101 kPa)	245
Melting point °C	111–114
pKa value	10.31
Stability	volatile with water vapour
Solubility g/l	0.3–0.7 in H ₂ O; highly soluble in organic solvents
Toxicity data	
Significantly less (~60%) than phenol (7.1.).	

Antimicrobial effectiveness/applications

o-Cymenol is effective against bacteria, moulds and yeast (see Table 57). In the EC list of preservatives for cosmetics the active agent is mentioned with a maximum allowed concentration of 0.1%. Percentage of use in US cosmetic formulations: 0.04%. Optimum pH range 4–9. Incompatible with nonionic and cationic surfactants.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.2. ALKYLPHENOLS
7.2.4. 5-Isopropyl-2-methylphenol
C₁₀H₁₄O



Molecular mass	150.22
CAS-No.	499-75-2
EC-No.	207-889-6
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	Carvacrol, Isothymol, 5-isopropyl-o-cresol, 2-methyl-5-(1-methylethyl)phenol
Supplier	MERCK, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	viscous colourless fluid which tends to become yellowish to orange by oxidation; its odour is similar to that of Thymol; Carvacrol is found in thyme, marjoram and oregano
Content (%)	~100
Boiling point/range °C (101 kPa)	237–238
Solidification point °C	3–3.5
Density g/ml (20°C)	0.976
Refractive index nD (20°C)	1.5233
Flash point °C	106.7
pKa value	10.32
Stability	volatile with water vapour; easy oxidizable
Solubility g/l (°C)	virtually insoluble in H ₂ O, highly soluble in organic solvents

Toxicity data

LD ₅₀ oral	810 mg/kg rat
intraperitoneal	73.3 mg/kg mouse
subcutaneous	680 mg/kg mouse
intravenous	89 mg/kg mouse

Rabbit skin exposed (24 h) to 500 mg; Severe irritation.

Antimicrobial effectiveness/applications

See under 7.2.2., Table 57

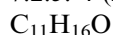
Microbicide group (substance class)

7.2. ALKYLPHENOLS

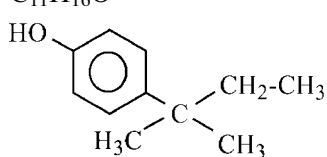
Chemical name

7.2.5. 4-(2-Methylbutyl)phenol

Chemical formula



Structural formula



Molecular mass

164.25

CAS-No.

80-46-6

EC-No.

201-280-9

TSCA Status

listed on the TSCA inventory list

Synonym/common name

p-tert. pentylphenol, p-tert.amylphenol (PTAP)

Supplier

SCHENECTADY INT. INC.

Chemical and physical properties

Appearance

colourless crystals with a phenolic odour

Content (%)

> 98

Boiling point/range °C (101 kPa)

265–267

Melting point °C

93–94

Density g/ml (20°C)

0.962

Flash point °C

121

Log POW

4.03

Stability

stable under normal conditions

Solubility g/l (20°C)

2 in H₂O; soluble in polar and non polar organic solvents

Toxicity data (source: SCHENECTADY INT. INC.)

LD ₅₀ oral	1830 mg/kg rat
dermal	2000 mg/kg rat

Corrosive to the skin and mucous membranes.

Ecotoxicity:

Aquatic fate: volatilizes slowly (half lives: river ~23 days; lakes ~73 days). Atmospheric fate: degrades photochemically (half life ~9h).

Acute fish toxicity: LC₅₀ for Fathead minnow 2.5 mg/l (96 h).

Table 58 Microbicidal concentrations within 10 min according to AOAC use-dilution method (Block, 1983)

Test organisms	Microbicidal conc. (g/litre)
<i>Pseudomonas aeruginosa</i>	> 1.0
<i>Salmonella choleraesuis</i>	0.4
<i>Staphylococcus aureus</i>	0.5

Antimicrobial effectiveness/applications

p-Tert. amylphenol is used as an active ingredient in disinfectants, in surface disinfectants, together with other microbicidal phenolic compounds which close the gap for Pseudomonads in the activity spectrum of p-tert.amylphenol.

Microbicide group (substance class)

7.2. ALKYLPHENOLS

Chemical name

7.2.6. Benzylphenols

Chemical formula

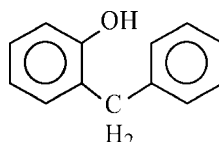
$C_{13}H_{12}O$

Molecular mass

184.23

Structural formula

7.2.6a. 2-Benzylphenol



CAS-No.

28944-41-4

EC-No.

249-361-8

Synonym/common name

2-phenylmethylphenol, 2-benzyl-1-hydroxy-benzene

Chemical and physical properties

Appearance

colourless crystals; tends to discolour to a moist pink and tan solid

Content %

~100

Boiling point/range °C (101 kPa)

312

Melting point °C

51.5–53

Flash point °C

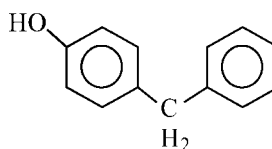
> 110

Solubility

sparingly soluble in H_2O , highly soluble in organic solvents

Structural formula

7.2.6b. 4-Benzylphenol



CAS-No.

101-53-1

EC-No.

unknown

Chemical and physical properties

Appearance

colourless crystals

Content %

~100

Boiling point/range °C (101 kPa)

325–330

Melting point °C

84

Solubility

sparingly soluble in H_2O , highly soluble in organic solvents

Toxicity data for a 40/60 mixture of 7.2.6a. and 7.2.6b.

LD₅₀ oral

3360 mg/kg rat

dermal

1.78 ml of a 80% solution in ethanol/kg rat

Irritant and corrosive to skin and mucous membranes.

Antimicrobial effectiveness/applications of the 40/60 mixture of 7.2.6a. and 7.2.6b.

The a.m. mixture of benzylphenols is used as an active ingredient in disinfectants, generally in combination with other active phenolics able to close the gap for Pseudomonads. The fact that the MICs (see Table 59) are higher than the microbicidal concentrations listed in Table 60 demonstrates the sensitivity of benzylphenols to organic matter. As a preservative for material protection the mixture has not gained importance because of unfavourable solubility properties.

Table 59 Minimum inhibition concentrations (MIC) of the benzyl-phenol mixture in nutrient agar

Test organism	MIC (mg/litre)
<i>Bacillus punctatus</i>	100
<i>Bacillus subtilis</i>	100
<i>Escherichia coli</i>	500
<i>Leuconostoc mesenteroides</i>	100
<i>Proteus vulgaris</i>	200
<i>Pseudomonas aeruginosa</i>	5000
<i>Pseudomonas fluorescens</i>	5000
<i>Staphylococcus aureus</i>	100
<i>Alternaria tenuis</i>	75
<i>Aspergillus flavus</i>	200
<i>Aspergillus niger</i>	100
<i>Aspergillus terreus</i>	200
<i>Aureobasidium pullulans</i>	100
<i>Candida albicans</i>	100
<i>Chaetomium globosum</i>	50
<i>Cladosporium herbarum</i>	200
<i>Coniophora puteana</i>	35
<i>Lentinus tigrinus</i>	75
<i>Paecilomyces variotii</i>	100
<i>Penicillium citrinum</i>	100
<i>Penicillium glaucum</i>	100
<i>Polyporus versicolor</i>	100
<i>Sclerophoma pityophila</i>	100
<i>Stachybotrys atra corda</i>	35
<i>Trichoderma viride</i>	200
<i>Trichophyton pedis</i>	20

Table 60 Microbicidal activity of the benzylphenol mixture within 10 min – use dilution test

Test organism	Microbicidal conc. (g/litre)
<i>Escherichia coli</i>	0.3
<i>Pseudomonas aeruginosa</i>	0.8
<i>Staphylococcus aureus</i>	0.25

Microbicide group (substance class)

Chemical name

Chemical formula

Molecular mass

Structural formula

CAS-No.

EC-No.

Synonym/common name

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

Melting point °C

pKa value

Solubility

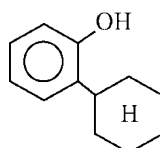
7.2. ALKYLPHENOLS

7.2.7. CYCLOHEXYLPHENOLS

 $C_{12}H_{16}O$

176.26

7.2.7a. 2-Cyclohexylphenol



119-42-6

unknown

1-cyclohexyl-2-hydroxy-benzene

colourless crystals

100

238

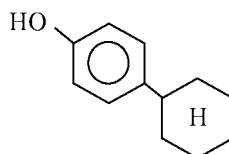
55.7

10.59

sparingly soluble in H_2O , highly soluble in organic solvents

7.2.7b. 4-Cyclohexylphenol

Structural formula



CAS-No.

1131-60-8

EC-No.

unknown

Chemical and physical properties

Appearance

colourless crystals

Content

100

Boiling point °C (101 kPa)

295

Melting point °C

128

pKa value

10.30

Solubility

sparingly soluble in H₂O, highly soluble in organic solvents*Antimicrobial effectiveness/applications*

The cyclohexylphenols are more active against moulds than against bacteria; but in total their activity spectrum is very much unequalized. This in connection with poor water solubility and unfavourable partition coefficients have up to now inhibited the practical application of the cyclohexylphenols as microbicides.

7.3. Halogenated alkylphenols

As a rule it has to be stated that halogenated alkylphenols are more active and broader in effectiveness than the alkylphenols. It is therefore in no way astonishing that some of the most important phenol derivatives in practical application are found in this class of phenolics. Among others Klarmann et al. (1933) have carried out systematic examinations of the relationship between chemical structure and antimicrobial activity with regard to halogenated alkylphenols. However, one should not overestimate the value of the data obtained when decisions and selections have to be made for practical application, as other properties of the microbicidal compounds, such as water-solubility, partition coefficient, activity in the presence of interfering factors encountered in practice, and toxicity, are of the same or of even higher importance. Although there are available a lot of data and experience, often the optimum compound and formulation must be determined by experiment.

Microbicide group (substance class)

7.3. HALOGENATED ALKYLPHENOLS

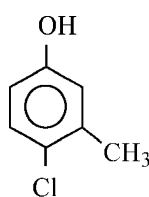
Chemical name

7.3.1. 4-Chloro-3-methylphenol

Chemical formula

C₇H₇ClO

Structural formula



Molecular mass

142.59

CAS-No.

59-50-7

EC-No.

200-431-6; EEC-no. 24

EPA-Reg.

approval for antimicrobial applications, including disinfectants

FDA

approval for use as a preservative for food contact applications

Synonym/common name

p-chloro-m-cresol (PCMC)

Supplier

BAYER AG

Chemical and physical properties

Appearance

white pellets with a distinct phenolic odour

Content %

> 99

Boiling point/range °C (101 kPa)	~235
Melting point °C	63–65
Density g/ml (20°C)	1.37
Bulk density kg/m ³	~630
Vapour pressure hPa (20°C)	0.08; 7 at 100°C
Flash point °C	~118
Auto ignition temperature °C	~590
pKa value	9.6
pH (0.1% in H ₂ O)	6.5
Log POW	3.02
Stability	volatile with water vapour; may become slightly discoloured when exposed to air, light, moisture or heat, stable over a wide pH range from 1 to 14
Solubility g/l (20°C)	~4 in H ₂ O (7 in H ₂ O at 50°C), 320 in 10% NaOH, 500 in ethanol
Toxicity data (source: BAYER AG)	
LD ₅₀ oral	1800–5000 mg/kg rat
dermal	> 2000 mg/kg rat
LC ₅₀ inhalation (dust, 4 h)	> 704 mg/m ³ air for rats (the concentration caused no deaths)

Not irritant on the rabbit skin (exposure 4 h), however corrosive to rabbit eyes. In the guinea pig test PCMC acts as skin sensitizer. Mutagenic effects were not observed, neither in the Salmonella/microsomes test nor in the micronucleus test on mice.

Ecotoxicity:

Tested according the OECD-Test Guideline 301 C PCMC is rated as “easily degradable” (> 90%); see Figure 15. The formation of stöchiometric amounts of chloride ions demonstrated the complete mineralisation of the product.

EC ₅₀ for activated sludge organisms	60 mg/l
EC ₅₀ for algae (<i>Scenedesmus</i> subs.)	> 10 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	2.29 mg/l (48 h)
LC ₅₀ for <i>Leuciscus idus</i>	1.2 mg/l (48 h)
LC ₅₀ for <i>Onchorhynchus mykiss</i>	0.9 mg/l (96 h)

Antimicrobial effectiveness/applications

PCMC is a microbicide well known for many years, with a long history of human exposure without any adverse effects. This fact is an advantage the importance of which one cannot overestimate; it is in line with the toxicity data which in the meantime have been very thoroughly investigated. As is demonstrated in Table 55 PCMC presents a broad and equalized spectrum of antimicrobial efficacy; it covers bacteria (including mycobacteria and Pseudomonads), yeasts and mould producing fungi (exception: some wood destroying basidiomycetes).

PCMC is most effective between pH 4 and 8, where, in fact, contrary to other phenol derivatives only PCMC is sufficiently water soluble. By comparing the microbicidal concentrations of PCMC with those of PCMC-Na (7.3.la.), solutions of which have pH values higher than 8, one notices a distinctive decrease in the activity of PCMC in alkaline media (Table 61).

Table 61 Microbicidal activity within 5 and 10 min of PCMC and PCMC-Na – use-dilution test

Test organism	Microbicidal concentration (g/litre)			
	PCMC		PCMC-Na	
	5 min	10 min	5 min	10 min
<i>Candida albicans</i>	1.0	1.0	6.0	6.0
<i>Escherichia coli</i>	1.5	1.0	8.0	4.0
<i>Pseudomonas aeruginosa</i>	1.5	0.7	6.0	6.0
<i>Staphylococcus aureus</i>	1.0	1.0	10.0	10.0

Especially remarkable is the high activity of PCMC against lipophilic, enveloped viruses, such as Herpes, simplex, Hepatitis B or HIV viruses. As other phenolic membrane active agents also PCMC exhibits a weak effect only on naked viruses and resistant bacterial spores (e.g. bacillus spores). However, a fully virucidal effect is achievable by combining PCMC formulations with small concentrations of glutaraldehyde (2.5.).

Because of its distinctive and reliable effectiveness, uninfluenced by detergents and heavy contamination, PCMC is a very important active ingredient in disinfectants for hospitals, public buildings, households and animal stables. The lethal concentrations of PCMC listed in Table 62 indicate the disinfectant effect of PCMC

Table 62 Bactericidal/disinfecting effect of PCMC (Source: BAYER AG)

Test organism	Contact time in minutes	Germ count germs/ml	Lethal active concentration in ppm PCMC			
			WHS		DW	
			without serum	with serum	without serum	with serum
Staphylococcus aureus	5–60	approx.10 ⁷	750–500	2500	1000–750	2500–1000
	5–60	approx.10 ³	250–100	1000–750	500–250	2500–1000
Escherichia coli	5–60	approx.10 ⁷	750–500	2500–750	1000–500	1000–750
	5–60	approx.10 ³	500	1000–750	1000–500	1000–500
Pseudomonas aeruginosa	5–60	approx.10 ⁷	1000–500	2500–750	2500–500	2500–750
	5–60	approx.10 ³	500	1000–750	500–250	1000–750

WSH = water of standardised hardness, 300 ppm CaCO₃

DW = demineralised water

without and with protein loading (10% albumin and 1% yeast extract) with two different water hardnesses in relation to different degrees of microbial contamination (germ counts). The tests were performed in accordance with the guideline of the German Association for Hygiene and Microbiology for qualitative suspension disinfection using a PCMC standard formulation of the following composition: 10% PCMC – 25% sec. alkane sulphonate – 20% isopropanol – 45% demineralised water.

In general higher PCMC concentrations are necessary to achieve a bactericidal effect against mycobacteria, e.g. 1500–5000 ppm for *Mycobacterium tuberculosis* ATCC 25618.

Due to their broad activity spectrum and favourable properties PCMC and its sodium salt are proven preservatives for the in-can/in-tank protection of very different functional fluids, e.g. solutions of thickeners and adhesives, pigment and filler slurries, concrete additives, textile and leather auxiliaries, paper coatings and metalworking fluids. Especially for the preservation of protein containing products PCMC is the first choice because of its compatibility with protein and its unbroken activity in the presence of protein. For the in-tank protection of metalworking fluids PCMC-Na is a very important supplement of formaldehyde releasing compounds as it is also highly effective against formaldehyde resistant bacteria. Problems in so-called bio-resistant lubricoolants, which exhibit bacteriostatic efficacy, are caused by uninhibited fungal growth leading to clogging of pipes and filters; such problems may be easily overcome by the application of PCMC or PCMC-Na, which are very active against a great variety of different species of fungi. The application of PCMC is also widespread in the leather industry, e.g. for the protection of wet blues.

In the EC positive list of preservatives allowed for the in-can protection of cosmetics PCMC is mentioned with a maximum authorized concentration of 0.2% and the limitation: prohibited in products intended to come into contact with mucous membranes.

Microbicide group (substance class)

Chemical name

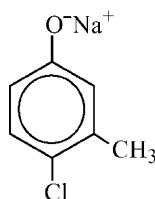
Chemical formula

Structural formula

7.3. HALOGENATED ALKYLPHENOLS

7.3.la. 4-Chloro-3-methylphenol, sodium salt

C₇H₇ClNaO·3.5mol H₂O



Molecular mass

227.63

CAS-No.

15733-22-9

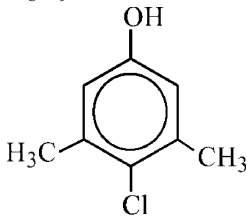
EC-No.	239-825-8
Registrations	see 7.3.1. (PCMC)
Synonym/common name	PCMC sodium salt, sodium-4-chloro-3-methylphenolate
Supplier	BAYER AG
Chemical and physical properties	
Appearance	practically odourless white to yellowish flakes
Content (%)	PCMC: ~63
Melting point °C	~94
Density g/ml (20°C)	1.36
Bulk density kg/m ³	~700
Vapour pressure hPa (100°C)	0.001
Auto ignition temperature °C	> 250
pH value (1 g/l H ₂ O)	10.5–11.5
Stability	may become slightly discoloured when exposed to air, light, moisture or heat
Solubility g/l (20°C)	580 in H ₂ O, ~2000 in ethanol, ~450 in isopropanol
Toxicity data (source: BAYER AG)	
LD ₅₀ oral	~1 610 mg/kg male rat ~1 360 mg/kg female rat

In connection with its alkalinity the PCMC sodium salt is severely corrosive to the skin, eyes and mucous membranes.

Ecotoxicity: The product is ready biodegradable. Its aquatic toxicity corresponds to its PCMC (7.3.1) content.

Antimicrobial effectiveness/applications

See PCMC (7.3.1.).

Microbicide group (substance class)	7.3. HALOGENATED ALKYLPHENOLS
Chemical name	7.3.2. 4-Chloro-3,5-dimethylphenol
Chemical formula	C ₈ H ₉ ClO
Structural formula	
Molecular mass	156.61
CAS-No.	88-04-0
EC-No.	201-793-8; EEC-no. 26
EPA-Reg.	Section 8(B) Chemical Inventory
Synonym/common name	p-chloro-m-xylenol; PCMX, 2-chloro-5-hydroxy-1,3-dimethylbenzene, chloroxylenol
Supplier	CLARIANT-NIPA, SIGMA
Chemical and physical properties	
Appearance	white crystals with slight smell of phenol
Content (%)	~100
Boiling point/range °C (101 kPa)	246
Melting point °C	114–116
Density g/ml (20°C)	0.67
Vapour pressure hPa (100°C)	3
Flash point	138
pKa value	9.7
pH (0.02% i. H ₂ O)	7
Stability	volatile with water steam
Solubility g/l (20°C)	0.33 i H ₂ O; readily soluble in alkaline solutions and organic solvents; soluble in fats and oils

Toxicity data

LD ₅₀ oral	3830 mg/kg rat 1000 mg/kg mouse
LC ₅₀ on inhalation	6.29 mg/l (4 h) for rats
LD ₅₀ intraperitoneal	115 mg/kg mouse

Moderately irritant to skin and mucosa. Low sensitization potential.

Not mutagenic (Ames test). Not teratogenic according to a test with rats.

Ecotoxicity (source: CLARIANT-NIPA): PCMX is not readily biodegradable (see Figure 15), but does not bioaccumulate.

LC ₅₀ for rainbow trout	0.77 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	7.7 mg/l (48 h)

Antimicrobial effectiveness/applications

The MICs in Table 55 show the efficiency of PCMX and the broadness of its activity spectrum. Starting from a pine oil based PCMX concentrate/disinfectant (pH 9) the PCMX concentrations listed in Table 62 proved microbicidal within 15 min. Due to its strong microbicidal activity PCMX is used as an active ingredient in disinfectants; however, one has to bear in mind that it is relatively sensitive to organic matter. Additionally PCMX is recommended as an active ingredient in deodorants, soaps, skin preparations for dermatological disorders, and antiseptics; the use level for such applications is normally in the range of 3–5%.

Moreover PCMX has gained some importance as a preservative for the protection of functional fluids, especially those containing protein, although the poor water solubility of PCMX does not particularly favour this application. The recommended addition rates move between 0.3–0.5% based on the total weight of finished product.

In the EC PCMX is listed among the preservatives allowed for the applications in cosmetics with a maximum authorized concentrations of 0.5%. – Percentage of use in US cosmetic formulations: 0.27%.

Table 63 Microbicidal PCMX concentration – use-dilution test

Test organism	Microbicidal PCMX concentration (g/litre)	
	In water	In water 'loaded with serum' ^a
<i>Escherichia coli</i>	0.2	0.5
<i>Proteus mirabilis</i>	0.2	0.5
<i>Pseudomonas aeruginosa</i>	0.1	2.0
<i>Staphylococcus aureus</i>	0.3	> 2.0

^a 20% bovine albumin.

Microbicide group (substance class)

Chemical name

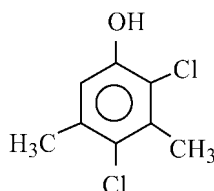
Chemical formula

Structural formula

7.3. HALOGENATED ALKYLPHENOLS

7.3.3. 2,4-Dichloro-3,5-dimethylphenol

C₈H₈Cl₂O



Molecular mass

CAS-No.

EC-No.

EPA TSCA

Synonym/common name

Supplier

191.06

133-53-9

205-109-9

Chemical Inventory

dichloro-m-xenol, DCMX, 2,4-dichloro-1-hydroxy-3,5-dimethylbenzene

CLARIANT-NIPA, MERCK

Chemical and physical properties

Appearance	crystalline solid with a distinctive phenolic odour
Content (%)	~100
Boiling point/range °C (101 kPa)	250
Melting point °C	95–96 (sublimation)
Flash point °C	134
Stability	stable under normal conditions, volatile with water steam
Solubility g/l (15°C)	0.2 in H ₂ O, 140 in benzene, 150 in toluene, 730 in acetone, 590 in diethylketone, 40 in chloroform; soluble in alkaline solutions

Toxicity data

LD ₅₀ oral	2810–4120 mg/kg rat

Antimicrobial effectiveness/applications

DCMX which is obtained by reaction of 4-chloro-3,5-dimethylphenol (PCMX; 7.3.2.) with N-chloroacetamide in glacial acetic acid plus concentrated HCl behaves similar to PCMX in activity and properties, but is of inferior importance in application as an active agent in pine oil based disinfectants or as a preservative for functional fluids, as it is even less soluble in water than PCMX and has a more intense phenolic smell.

Table 64 Minimum inhibition concentrations (MIC) of DCMX in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	2000
<i>Pseudomonas aeruginosa</i>	1000
<i>Staphylococcus aureus</i>	50
<i>Aspergillus niger</i>	200
<i>Chaetomium globosum</i>	200
<i>Penicillium glaucum</i>	100

Microbicide group (substance class)

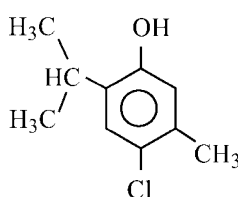
Chemical name

Chemical formula

Structural formula

7.3. HALOGENATED ALKYLPHENOLS

7.3.4. 4-Chloro-6-isopropyl-3-methylphenol

C₁₀H₁₃ClO

Molecular mass

184.67

CAS-No.

89-68-9

EC-No.

201-930-1

Synonym/common name

Chlorthymol, 4-chloro-1-hydroxy-2-isopropyl-5-methylbenzene

Chemical and physical properties

Appearance	colourless crystals with a pleasant odour similar to Thymol (7.2.2.)
Content (%)	~100
Boiling point/range °C (101 kPa)	259–263
Melting point °C	64
Stability	volatile with water vapour
Solubility g/l (20°C)	0.3 in H ₂ O; soluble in alkaline solutions, highly soluble in organic solvents

Toxicity data

LD₅₀ oral

2.460 mg/kg mouse

Irritant to skin and mucous membranes.

Antimicrobial effectiveness/applications

Apparently 4-chloro-thymol is in particular effective against mould producing fungi, but less active against bacteria and insufficient in activity against Pseudomonads. 4-Chloro-thymol has up to now not gained importance as a preservative or an active ingredient in disinfectants. However, it is used, as well as Chlorocarvacrol, the chlorination product of Carvacrol (7.2.4.), a viscous fluid with a faint odour (b.p. at 7 kPa 158°C), in pharmaceutical disinfectants.

Table 65 Minimum inhibition concentrations (MIC) of 4-chloro-thymol in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	200
<i>Pseudomonas aeruginosa</i>	4000
<i>Aspergillus niger</i>	100
<i>Chaetomium globosum</i>	20–50
<i>Penicillium glaucum</i>	50
<i>Rhizopus nigricans</i>	20–50

Table 66 Microbicidal concentrations of 4-chloro-thymol – use-dilution test

Test organism	Microbicidal concentration (g/litre) after 10 min exposure
<i>Escherichia coli</i>	0.2
<i>Pseudomonas aeruginosa</i>	> 20
<i>Staphylococcus aureus</i>	0.075

Microbicide group (substance class)

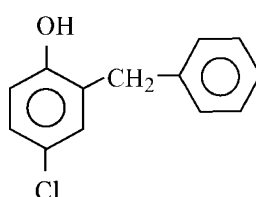
Chemical name

Chemical formula

Structural formula

7.3. HALOGENATED ALKYLPHENOLS

7.3.5. 2-Benzyl-4-chlorophenol

C₁₃H₁₁ClO

Molecular mass

218.69

CAS-No.

120-32-1

EC-No.

204-385-8; EEC-no. 40

EPA/FIFRA-Reg.

approval for antimicrobial applications

MITI-Reg.

No. 4-98

Synonym/common name

Chlorophen, 2-benzyl-4-chloro-1-hydroxybenzene

Supplier

BAYER AG

Chemical and physical properties

Appearance

colourless to yellowish flakes or solidified melt with phenolic odour

Content %

> 95

Boiling point/range °C (101 kPa)

327 (190–196 at 1.5 kPa)

Melting point °C	48–49
Density g/ml (20°C)	~1.2
Vapour pressure hPa (100°C)	0.1
Flash point °C	~188
Auto ignition temperature °C	~490
Log POW	4.4
pH value at 22.5°C	5.3 in saturated aqueous solution
Ionicity	anionic
pKa value	9.7
Stability	stable between pH 1 to 14
Solubility g/l (20°C)	0.5 in H ₂ O, > 3000 in ethanol, 1000 in 10% NaOH solution

Toxicity data (source: BAYER AG)

LD ₅₀ oral	> 5000 mg/kg rat
dermal	> 2500 mg/kg rat

Slightly irritant to the skin of rabbits (exposure: 4 h); severe irritation of rabbit eyes; cauterizes the cornea.

Ecotoxicity:

Chlorophen is rated as moderately degradable (closed bottle test/BOD-determination). See also Figure 15. The degradation activity of activated sludge organisms is not impaired at concentrations of 10 mg/l.

EC ₅₀ for activated sludge organisms	59.6 mg/l
LC ₀ for <i>Leuciscus idus</i>	~0.5 mg/l (48 h)
LC ₅₀ for <i>Brachydanio rerio</i>	~1.5 mg/l (96 h)

Table 67 MIC's (mg/l) of chlorophen for bacteria, yeast and mould fungi
(Source: BAYER AG)

Bacteria	
<i>Aeromonas punctata</i>	100
<i>Bacillus mycoides</i>	20
<i>Bacillus subtilis</i>	10
<i>Desulfovibrio desulfuricans</i>	50
<i>Enterobacter aerogenes</i>	20
<i>Escherichia coli</i>	1000–2000
<i>Escherichia coli</i> EHEC DSM 8579	500–1000
<i>Legionella pneumophila</i> ATCC 33152	100–200
<i>Leuconostoc mesenteroides</i>	10
<i>Listeria monocytogenes</i> DSM 20600	100
<i>Mycobacterium terrae</i> DSM 43227	100
<i>Propionibacterium acnes</i> DSM 20458	10–100
<i>Proteus mirabilis</i>	100–500
<i>Pseudomonas aeruginosa</i>	5000
<i>Pseudomonas fluorescens</i>	> 5000
<i>Salmonella choleraesuis</i> DSM 4224	1000–2000
<i>Staphylococcus aureus</i> MRSA	100
<i>Staphylococcus aureus</i> MRSA DSM 2569	100
Yeast	
<i>Candida albicans</i>	50
<i>Candida krusei</i>	100
<i>Rhodotorula mucilaginosa</i>	50
<i>Saccharomyces cerevisiae</i>	50
<i>Saccharomyces bailii</i>	20
<i>Torula rubra</i>	50
<i>Torula utilis</i>	35
Mould fungi	
<i>Alternaria alternata</i>	100–200
<i>Aspergillus flavus</i>	75
<i>Aspergillus niger</i>	50–100
<i>Aspergillus terreus</i>	100
<i>Aspergillus ustus</i>	500
<i>Chaetomium globosum</i>	20
<i>Microsporum canis</i>	5–10
<i>Mucor racemosus</i>	50
<i>Penicillium glaucum</i>	35–50
<i>Rhizopus stolonifer</i>	50
<i>Trichophyton mentagrophytes</i>	5–10
<i>Trichophyton rubrum</i>	5–10

Table 68 Microbicidal concentrations of Chlorophen within 5 min – use-dilution test

Test organism (approx. 10^7 /ml)	Microbicidal concentration (g/litre)	
	In demineralized water	Plus 'serum' ^a
<i>Escherichia coli</i>	0.1	1.0
<i>Pseudomonas aeruginosa</i>	10	25
<i>Staphylococcus aureus</i>	0.25	1.0

^a The serum load consists of 10% albumin + 10% yeast extract.

Antimicrobial effectiveness/applications

Table 67 lists the minimum inhibition concentrations (MIC) in mg/l of Chlorophen in nutrient agar contaminated with various test organisms.

Apparently the activity spectrum of Chlorophen against fungi and yeast is more equalized than that against bacteria which shows deep gaps for Pseudomonads. In disinfectants, the main application field for Chlorophen, it therefore should be used in combination with other active phenolic compounds, e.g. with OPP (7.4.1.), PCMC (7.3.1.), PCMX (7.3.2.), which close the gaps. That way it is possible to reduce the concentration of Chlorophen in disinfectants and to minimize the risk of skin irritation through Chlorophen. Such combinations additionally exhibit high efficacy against cocci and lipophilic, enveloped viruses. However, naked viruses and resistant bacteria spores are more or less resistant to phenolic microbicides.

Chlorophen works best in an acidic, neutral or weakly alkaline environment, where it is in the undissociated effective form. Optimum pH range 4 to 8. Chlorophen is compatible with anionic surfactants and soaps and to a certain extent with non-ionic surface active compounds, too, however, not with cationic surfactants. Starting from a concentrate, containing 10% Chlorophen + 25% sec. alkane sulphonate + 20% isopropanol + 45% demineralized water, Chlorophen exhibits microbicidal effects in water at concentrations listed in Table 68.

The unequaled activity spectrum has inhibited Chlorophen from gaining importance as a preservative for material protection. Nevertheless it is listed in the EC list of preservatives allowed for the incorporation into cosmetic products (maximum allowed concentration 0.2%).

Microbicide group (substance class)

Chemical name

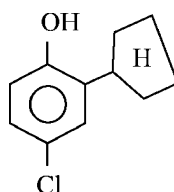
Chemical formula

Structural formula

7.3. HALOGENATED ALKYLPHENOLS

7.3.6. 4-Chloro-2-cyclopentylphenol

$C_{11}H_{13}ClO$



Molecular mass

196.68

CAS-No.

13347-42-7

EC-No.

unknown

Synonym/common name

1-hydrox-2-cyclopentyl-4-chlorobenzene

Chemical and physical properties

Appearance

brownish fluid with a characteristic odour

Content (%)

~100

Boiling point/range °C (101 kPa)

303

Melting point °C

23.7

Stability

sensitive to light and heat: increasing discolouration

Solubility g/l (20°C)

0.24 in H_2O ; is readily soluble in organic solvents

Toxicity data

LD₅₀ oral

2460 mg/kg rat

420 mg/kg rabbit

LD₅₀ dermal

850 mg/kg rabbit

Corrosive to skin and mucosa; 5% solutions cause still irritations.

Antimicrobial effectiveness/applications

4-chloro-2-cyclopentylphenol which is prepared by shaking 4-chlorophenol (7.5.1.) and cyclopentene in concentrated sulfuric acid is very effective against fungi (MIC 10–50 mg/l) and Staphylococci and Salmonella (microbicidal concentrations after 10 min: 400 mg/l), but insufficiently active against Pseudomonads. However, due to its toxicological properties it is no longer used as an active ingredient in disinfectants.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility g/l (20°C)

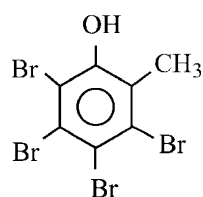
Toxicity data

LD₅₀ oral

Remarkably compatible with skin, mucosa and eyes.

7.3. HALOGENATED ALKYLPHENOLS

7.3.7. 2-Methyl-3,4,5,6-tetrabromophenol

C₇H₄Br₄O

423.75

576-55-6

209-403-8

tetrabromo-o-cresol

slightly yellowish, odourless, crystalline powder

~100

205–208

discolours in light and in contact with traces of iron
85 in ethanol, 80 in isopropanol, 9 in ethylene glycol,
8 in 1,2-propylene glycol, 4 in vaseline oil

> 6400 mg/kg rat

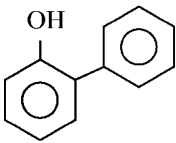
Antimicrobial effectiveness/applications

The compound is very effective against Staphylococci (MIC: approx. 2.5 mg/litre), but very much less active against Pseudomonads (MIC: 2000 mg/litre). Remarkable is the efficacy of tetrabromo-o-cresol against algae (MIC for Chlorella species: 1 mg/litre).

It has been proposed to use the compound as a preservative for cosmetics or as an active ingredient in deodorants or for the antimycotic treatment of textiles. However, the significance of tetrabromo-o-cresol has remained very limited (probably due to poor water-solubility, coloration risks and costs).

Table 69 Minimum inhibition concentrations (MIC) of tetrabromo-o-cresol for fungi in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	3000
<i>Chaetomium globosum</i>	60
<i>Penicillium glaucum</i>	2500
<i>Rhizopus nigricans</i>	1200

Microbicide group (substance class)	7.4. BIPHENYLOLS
Chemical name	7.4.1. 2-Biphenylol
Chemical formula	$C_{12}H_{10}O$
Structural formula	
Molecular mass	170.21
CAS-No.	90-43-7
EC-No.	201-993-5; EEC-no. 7
E-No.	231 meets the purity standards for food additives specified in EC Directives
EPA/FDA	approval for antimicrobial applications
Synonym/common name	2-phenylphenol, o-phenylphenol, OPP
Supplier	BAYER AG, DOW CHEM. CO.
Chemical and physical properties	
Appearance	colourless flakes or pale yellow crystalline mass with a slight phenolic odour
Content (%)	» 99.5
Boiling point/range °C (101 kPa)	286; (152–154°C at 2 kPa)
Melting point °C	56–58
Density g/ml (20°C)	1.26
Vapour pressure hPa (20°C)	7.10^{-4} ; (1 hPa at 100°C)
Viscosity mPa·s (100°C)	2.4
Flash point °C	~138
Auto ignition temperature °C	> 520
Upper flammability limit % v/v i.air	9.5
Lower flammability limit % v/v i.air	1.4
Log POW	3.18
pH of a saturated solution in H ₂ O	~6.1 at 22.7°C
pKa value	~11.6
Stability	stable between pH 1–14; volatile with water vapour
Solubility g/l (20°C)	0.2 in H ₂ O; 500 in 10% NaOH, 5900 in ethanol, 3300 in isopropanol, 2750 in ethylene glycol, 3000 in 1,2-propylene glycol, 6600 in acetone, 200 in white spirit, 2000 in pine oil
Toxicity data (source: BAYER AG)	
LD ₅₀ oral	2980 mg/kg rat
dermal	> 2000 mg/kg rat
Inhalation hazard test (dynamic vaporization), 14 days of further observation: rats, 7 h full body exposure: no symptoms were observed.	
The concentrated product is moderately irritant to the skin of rabbits (exposure 4 h), however, severely, irritant to rabbit eyes; a 0.1% aqueous solutions is non-irritant, neither to the skin nor to the eyes.	
Ecotoxicity:	
The product is ready biodegradable (see Figure 15).	
EC ₅₀ for activated sludge organisms	approx. 62.2 mg/l
EC ₅₀ for <i>Daphnia magna</i>	approx. 0.38 mg/l (48 h)
EC ₅₀ for <i>Daphnia magna</i>	1.5 mg/l (24 h)
LC ₅₀ for <i>Leuciscus idus</i>	20 mg/l (96 h)
for <i>Brachydanio rerio</i>	approx. 2.3 mg/l (96 h)
EC ₅₀ for <i>Scenedesmus subspicatus</i> (green alga)	0.85 mg/l (72 h)

Antimicrobial effectiveness/applications

The minimum inhibition concentrations (MIC) listed in the following Table demonstrate the broad activity spectrum of OPP.

Table 70 MIC (mg/l) of OPP in nutrient agar contaminated with various test organisms (Source: BAYER AG)

Bacteria	
<i>Aeromonas punctata</i>	200
<i>Bacillus mycoides</i>	100–300
<i>Bacillus subtilis</i>	100–200
<i>Desulfovibrio desulfuricans</i>	50
<i>Enterobacter aerogenes</i>	200
<i>Escherichia coli</i>	200
<i>Leuconostoc mesenteroides</i>	100–200
<i>Proteus mirabilis</i>	200
<i>Pseudomonas aeruginosa</i>	1500
<i>Pseudomonas fluorescens</i>	1000
<i>Staphylococcus aureus</i>	200–300
Yeasts	
<i>Candida albicans</i>	100
<i>Candida krusei</i>	200
<i>Rhodotorula mucilaginosa</i>	100
<i>Saccharomyces cerevisiae</i>	200
<i>Saccharomyces bailii</i>	100
<i>Rhodotorula rubra</i>	100
<i>Torula utilis</i>	100–200
Mould fungi	
<i>Alternaria tenuis</i>	100–200
<i>Aspergillus flavus</i>	100
<i>Aspergillus niger</i>	50–100
<i>Aspergillus terreus</i>	200
<i>Aspergillus ustus</i>	150
<i>Chaetomium globosum</i>	50–100
<i>Mucor racemosus</i>	200
<i>Penicillium brevicaulis</i>	50–100
<i>Rhizopus stolonifer</i>	200
<i>Trichophyton mentagrophytes</i>	50

Due to the fact that OPP is really a broad spectrum microbicide and because of its favourable toxicity and ecotoxicity data it has become one of the most important microbicides which is used in numerous different applications. It is an important active ingredient in disinfectants for hospitals, public buildings, households and animal stables. Preferably OPP is used for heavy duty disinfection where there is a high risk of infection or of the presence of strong impurities, or wherever water with a high hardness is used. As a membrane active microbicide OPP performs best in an acidic, neutral or weakly basic environment, where it is in its undissociated form. Very often OPP is formulated in combination with other phenolic microbicides, e.g. with Chlorophen (7.3.5.) and/or PCMC (7.3.1.). Worthy of note is that such combinations exhibit virucidal efficacy against lipophilic, masked viruses.

Disinfectant formulations based on OPP take the poor water solubility of OPP into consideration. Anionic surfactants and soaps are used preferably; to a limited extent (below the critical micellization concentration) non-ionic surfactants may be used, too. The rule that the microbicidal effect of phenolic compounds is dependent on such factors as the pH of the ready-to-use dilution, degree of contamination/germ count, water hardness and dirt content applies to OPP, too. The bactericidal effect of a standard formulations (SF) containing 10% OPP + 25% sec. alkanesulphonate + 20% isopropanol + 45% demineralized water is documented in Table 71 by the lethal active concentrations of OPP (mg/l) for various species of bacteria in dependence of pH, on protein loading and on a high germ count (approx. 10^7 germs per ml solution to be disinfected).

As a preservative for functional fluids OPP is used in adhesives, thickeners, lubricoolants, textile, leather and paper auxiliaries, ceramic glazes etc. Textile material, leather and paper is protected against biodeterioration through application of OPP or OPP-Na (7.4.1a.). It is gaining more and more importance as a substitute for PCP (7.5.4.) for the temporary protection of freshly cut wood and sawn timber. Even post harvest preservatives to prevent spoilage of stored fruits (e.g. citrus fruits) may be based on OPP; for the latter application the following limit is fixed in the USA and the EC: 10 mg/kg citrus fruit.

In the EC positive list of preservatives which cosmetic products may contain OPP and OPP sodium salt (7.4.1a.) are listed with a minimum authorized concentration of 0.2% (expressed as OPP). – Percentage of use in US cosmetic formulations: OPP 0.05%; OPP sodium salt 0.02%.

Table 71 Bactericidal concentrations of OPP ex SF at a germ count of 10^7 cfu/ml (Source: BAYER AG)

Test organism	Time in minutes	Lethal active concentration in mg OPP/l			
		WSH, pH 4		WSH, pH 8	
		without serum	with serum	without serum	with serum
<i>Staphylococcus aureus</i>	5–60	250–100	2500	750–250	2500–1000
<i>Escherichia coli</i>	5–60	500–100	1000–500	1000–250	1000–500
<i>Pseudomonas aeruginosa</i>	5–60	250–75	2500–500	1000	7500–2500

WSH = water of standardized hardness, 300 ppm CaCO_3

Microbicide group (substance class)

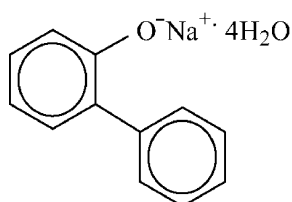
Chemical name

Chemical formula

Structural formula

7.4. BIPHENYLOLS

7.4.1a. 2-Biphenylol sodium salt as tetrahydrate

 $\text{C}_{12}\text{H}_9\text{NaO} \cdot 4\text{H}_2\text{O}$ 

Molecular mass

264.3

CAS-No.

132-27-4

EC-No.

205-055-6

E-No.

232, meets the purity standards for food additives specified in EC Directives

EPA/FDA

approval for antimicrobial applications

Synonym/common name

sodium-2-phenylphenolate containing 4 moles H_2O of crystallisation

Supplier

BAYER AG, DOW CHEM. CO.

Chemical and physical properties

Appearance

white to pale yellow flakes, odourless; a white to slightly reddish powder is another supply form

Content (%)

min. 95 (OPP content ~64)

Bulk density g/l (20°C)

400–450

pH (2% in water)

11.1–11.8

Stability

stable in solutions up to pH 14

Solubility g/l (20°C)

2000 in ethanol, 1500 in isopropanol; at 25°C: 1200 in H_2O , 3300 in acetone, > 3000 in ethylene glycol

Toxicity data (source: DOW.CHEM.CO.)

LD₅₀ oral

846 mg/kg male rat

591 mg/kg female rat

In consequence of its alkalinity the product is corrosive to skin, mucous membranes and eyes; corneal injury may result in permanent impairment of vision. Inhalation may cause irritation to upper respiratory tract.

No sensitization in the guinea pig test.

Mutagenicity studies were negative. – Non-teratogenic. – Carcinogenicity cannot be excluded.

Exposure limits (occupational) TWA-8 h for OPP 1 mg/m^3
 TLV for NaOH 2 mg/m^3

Ecotoxicity:

Passes OECD Test(s) for ready biodegradability.

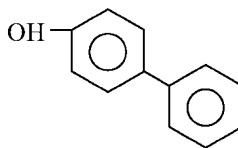
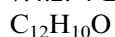
LC₅₀ for fathead minnow (*Pimephales promelas*) 6.0 mg/l LC₅₀ for *Daphnia magna* 3.8 mg/l *Antimicrobial effectiveness/applications*

See o-phenylphenol = OPP (7.4.1.).

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.4. BIPHENYLOLS

7.4.2. 4-Biphenylol



Molecular mass 170.21
CAS-No. 92-69-3
EC-No. 202-179-2
EPA TSCA Test submission Data Base, Jan. 2001
Synonym/common name 4-phenylphenol, p-phenylphenol, PPP
Supplier SIGMA-ALDRICH

Chemical and physical properties

Appearance white crystals
Content (%) ~100
Boiling point/range °C (101 kPa) 321
Melting point °C 165
Density g/ml (20°C) 1.219
Flash point °C 160
Stability stable between pH 1–14; volatile with water vapour
Solubility g/l (20°C) < 0.05 in H₂O, 0.1 in 10% NaOH, highly soluble in organic solvents

Toxicity data

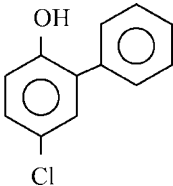
LD₅₀ intraperitoneal 150 mg/kg mouse
Irritant to skin, mucous membranes and eyes.

Antimicrobial effectiveness/applications

The pressure hydrolysis of chlorobenzene under alkaline conditions to phenol gives OPP and PPP as by-products (5–6%). The ratio of OPP/PPP is 2/1. Separation by fractional distillation is possible. Although the efficacy and activity spectrum of PPP are similar to those of OPP (7.4.1.), PPP has no importance as a microbicide for material protection or as an active ingredient in disinfectants. The reasons for this are the unfavourable solubility properties of PPP and the difficulty of providing PPP in larger quantities at reasonable costs.

Table 72 Minimum inhibition concentrations (MIC) of PPP in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	150
<i>Bacillus mycoides</i>	75
<i>Bacillus punctatum</i>	75
<i>Bacillus subtilis</i>	100
<i>Escherichia coli</i>	> 1000
<i>Leuconostoc measenteroides</i>	50
<i>Pseudomonas aeruginosa</i>	> 1000
<i>Pseudomonas fluorescens</i>	> 1000
<i>Proteus mirabilis</i>	> 1000
<i>Staphylococcus aureus</i>	50
<i>Aspergillus niger</i>	100
<i>Aureobasidium pullulans</i>	150
<i>Chaetomium globosum</i>	50
<i>Cladosporium cladosporioides</i>	50
<i>Lentinus tigrinus</i>	200
<i>Penicillium glaucum</i>	50–100
<i>Sclerophoma pityophila</i>	200
<i>Trichoderma viride</i>	200

Microbicide group (substance class)	7.4. BIPHENYLOLS
Chemical name	7.4.3. 4-Chloro-2-hydroxybiphenyl
Chemical formula	C ₁₂ H ₉ ClO
Structural formula	
Molecular mass	204.66
CAS-No.	1331-46-0
EC-No.	unknown
Synonym/common name	4-Chloro-2-phenylphenol, monochloro-o-phenylphenol, MCOPP
Chemical and physical properties	
Appearance	white crystals; the technical product is a pale straw coloured liquid containing 80% MCOPP, 15% of the 6-chloro-isomer and 5% other chlorinated OPP derivatives
Content %	~100
Boiling point/range °C (100 kPa)	250–300 (178°C at 1.5 kPa)
Melting point °C	36–37
Solubility	sparingly soluble in water, highly soluble in organic solvents
Toxicity data	
LD ₅₀ oral	3500 mg/kg rat
Irritant and corrosive to skin, mucosa and eyes, may cause contact dermatitis. Percutaneously toxic.	

Antimicrobial effectiveness/applications

MCOPP is much more effective than Chlorophen (7.3.5.) and was used in pine oil/soap based disinfectants as an active ingredient. However, the unfavourable toxicological properties of MCOPP have led to this application being discontinued.

Table 73 Microbicidal concentrations of MCOPP in water – use-dilution test

Test organism	Microbicidal conc. (g/litre)
<i>Escherichia coli</i>	0.05
<i>Pseudomonas aeruginosa</i>	2.0
<i>Staphylococcus aureus</i>	0.05

7.5. Halogenated Phenols

The halogenation of phenol, preferably its chlorination, produces halogen phenols that possess a far higher anti-microbial effect and a far higher acidity than the basic product. But halogenating phenol means not only obtaining microbicides of better efficacy, but also of higher persistence, the degree of persistence increasing with the degree of chlorination. Among the microbicides for the protection of materials, chlorinated phenol derivatives, i.e. tri-, tetra- and above all penta-chlorophenol, therefore did play a leading role in the past because they largely came up to the (hardly differentiated) demands that were made on microbicides until a few years ago, viz.

- powerful effect and broad effective spectrum,
- highest possible stability = persistence,
- economical formulation.

In the meantime such requirements are out of date or have become more differentiated. The demand is no longer for persistence at any price, but for limited persistence graduated according to the application, for stability or instability as appropriate. Nor is there necessarily a demand for microbicides with so wide a range of effectiveness as pentachlorophenol – fungicidal, bactericidal, algicidal, insecticidal, molluscicidal, herbicidal (which therefore should better be referred to as 'biocides'). What is required are such microbicides that display the desired – for instance fungicidal – activity, and no more, at very low concentrations. The toxicity of polychlorinated phenol derivatives increases with the degree of chlorination. On account of their toxicity and ecotoxicity, polychlorinated phenol derivatives are very liable to be substituted by other products, their use for the protection of materials being more and more on the decrease. However, regarding the ecotoxicity of the highly chlorinated phenols such as 2,4,6-trichlorophenol (7.5.2.), 2,4,5-trichlorophenol (7.5.3.) and pentachlorophenol (7.5.4.) it is often forgotten, that they are not totally resistant to biodegradation. According to a review of Neilson (1996) these phenol derivatives may be subject to degradation by initial loss of chloro ions before fission of the aromatic ring.

On account of their lessening importance only the main representatives of polychlorinated phenols will be the subject of the following brief description.

Microbicide group (substance class)

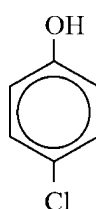
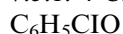
Chemical name

Chemical formula

Structural formula

7.5. HALOGENATED PHENOLS

7.5.1. 4-Chlorophenol



Molecular mass

128.56

CAS-No.

106-48-9

EC-No.

203-402-6

EPA TSCATS

January 2001

Synonym/common name

p-chlorophenol, 4-hydroxychlorobenzene

Supplier

SIGMA-ALDRICH

Chemical and physical properties

Appearance

crystals with typical phenolic odour

Content (%)

> 99

Boiling point/range °C (101 kPa)

220 (101–102 at 1.5 kPa)

Melting point °C

43

Density g/ml (20°C)

1.26

Vapour pressure hPa (20°C)

1.33

Flash point °C

121

Stability

volatile with water vapour

Solubility

sparingly soluble in H₂O, highly soluble in ethanol, glycerol, ether, chloroform, fats and ethereal oils

Toxicity data

LD₅₀ oral

670 mg/kg rat

dermal

1500 mg/kg rat

subcutaneous

1030 mg/kg rat

intraperitoneal

281 mg/kg rat

LD₅₀ inhalative

11 mg/m³ for rats

Severe irritation of the rabbit skin (2 mg/24 h) and the rabbit eyes (0.25 mg/24 h).

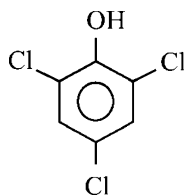
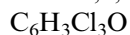
Antimicrobial effectiveness/applications

4-Chlorophenol which is easily synthesized by chlorination of phenol (7.1) in the cold exhibits considerable antibacterial and antifungal activity. However, these properties are practically of no avail in consideration of the high toxicity and the strong adhering odour of the substance.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.5. HALOGENATED PHENOLS

7.5.2. 2,4,6-Trichlorophenol



Molecular mass
CAS-No.
EC-No.
EPA TSCATS
Synonym/common name
Supplier

197.45
88-06-2
201-795-9
Data Base, Jan. 2001
1-hydroxy-2,4,6-trichlorobenzene
FLUKA CHEMIE

Chemical and physical properties

Appearance

orange-brown crystalline chunks with a distinctive phenolic odour

Content (%)

~97

Boiling point/range °C (101 kPa)

246

Melting point °C

64–66

Density g/ml (75°C)

1.4901

Vapour pressure hPa (20°C)

0.0073

pKa value at 25°C

8.5

Stability

stable under normal conditions; with NaOH sodium-2,4,6-trichlorophenolate is formed (CAS-No. 3784-03-0, EC-No. 223-246-2) which is readily soluble in H₂O

Solubility g/l (25°C)

0.9 in H₂O; soluble in alkaline solutions, readily soluble in organic solvents

Toxicity data

LD₅₀ oral

820 mg/kg rat
1000 mg/kg guinea pig

LD₅₀ intraperitoneal

276 mg/kg rat

Moderately to severely irritant to skin, mucosa and eyes.

Limited evidence to reasonable anticipation to be carcinogenic.

Ecotoxicity:

Highly toxic for fish (LC₀ < 0.5 mg/l) and activated sludge organisms.

González et al. (1996) found that *Alcaligenes eutrophus* is capable for the degradation of 2,4,6-trichlorophenol.

Antimicrobial effectiveness/applications

Minimum inhibition concentrations of 2,4,6-trichloro-phenol in nutrient media (mg/litre):

For bacteria

approx. 500–100

For fungi

approx. 20

For algae

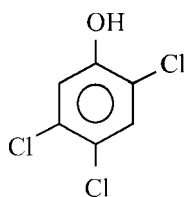
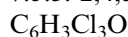
approx. 20–30

The microbicidal activity is considerably reduced in the presence of organic matter. Much more than for other phenol derivatives the efficacy of trichlorophenols depends on pH variations. 2,4,6-trichlorophenol also exhibits insecticidal effectiveness. Nowadays it is still used as an active ingredient in wood preservatives especially for the anti-sapstain treatment of freshly cut and sawn timber, moreover in preservatives for the leather industry and for functional technical fluids, e.g. protein containing formulations, concrete additives. But altogether the product as a microbicide is on the decline because of its toxicity/ecotoxicity and its intensive adherent phenolic odour.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

7.5. HALOGENATED PHENOLS

7.5.3. 2,4,5-Trichlorophenol



Molecular mass
CAS-No.
EC-No.
EPA TSCATS
Synonym/common name
Supplier

197.45
95-95-4
202-467-8
Data Base, Jan 2001
1-hydroxy-2,4,6-trichlorobenzene
FLUKA CHEMIE

Chemical and physical properties

Appearance

white to tan crystalline flakes with an extremely intensive and adherent phenolic odour

Content (%)

~100

Boiling point/range °C (98,5 kPa)

248

Melting point °C

67–69

Stability

stable under normal conditions

Solubility

sparingly soluble in H_2O , readily soluble in organic solvents, soluble in sodium hydroxide solution under formation of sodium 2,4,5-trichlorophenolate

Toxicity data

LD₅₀ oral
intrapertitoneal
subcutane

820 mg/kg rat

355 mg/kg rat

2260 mg/kg rat

LD₅₀ oral
intravenous

600 mg/kg mouse

56 mg/kg mouse

Irritant to skin, mucosa and eyes.

With regard to carcinogenicity, evidence is inadequate according to IARC monographs on the evaluation of the carcinogenic risk of chemicals to man (WHO International Agency for Research on Cancer).

Exposure limit (occupational) Denmark/Germany 0.5 mg/m³.

Ecotoxicity:

Highly toxic for fish (LC₀ < 0.5 mg/l) and activated sludge organisms.

Antimicrobial effectiveness/applications

Minimum inhibition concentrations in nutrient media (mg/litre):

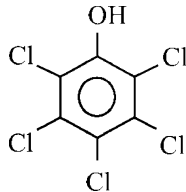
For bacteria

50–250

For fungi

10

Apparently 2,4,5-trichlorophenol is, compared with 2,4,6-trichlorophenol, the more potent microbicide. It has found application in agents for the preservation of wood and leather (wet blues) and in preservatives for functional technical fluids, especially in protein containing products, e.g. animal glues. In the meantime production and use as a microbicide has been stopped almost completely, mainly because of the risk of forming 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) during production and burning of 2,4,5-tetrachloro-phenol. TCDD, the 'Seveso poison' is an extremely toxic compound whose LD₅₀ to the guinea pig is 600 ng/kg. Laterally substituted congeners containing altogether 4, 5 or 6 chlorine atoms are highly toxic to fish, particularly to the early life stages of fish, with reported effective concentrations in the ng/l range. These congeners are also the most readily bioaccumulated. An aquatic toxicity threshold concentration of 0.011–0.038 ng/l, applicable to natural ecosystems has been determined for 2,4,7,8-tetrachlorodibenzo-*p*-dioxin (or for mixtures of congeners expressed as 2,3,7,8-TCDD equivalents) (Grimwood, Dobbs, 1995).

Microbicide group (substance class)	7.5. HALOGENATED PHENOLS
Chemical name	7.5.4. Pentachlorophenol (PCP)
Chemical formula	C_6HCl_5O
Structural formula	
Molecular mass	266.34
CAS-No.	87-86-5
EC-No.	201-778-6
Synonym/common name	1-hydroxy-pentachlorobenzene
Chemical and physical properties	
Appearance	white to grey crystalline powder or flakes with phenolic odour
Content (%)	~99
Boiling point/range °C (101 kPa)	310 (decomposition)
Melting point °C	191
Density g/ml (191°C)	1.978
Vapour pressure hPa (20°C)	5.1×10^{-5} (0.16 at 100°C)
pKa value	5.26
Stability	very stable under normal conditions
Solubility g/l (20°C)	0.02 in H_2O ; soluble in organic solvents and alkaline media; the sodium salt, PCP-Na hydrate (CAS-No. 131-52-2, EC-No. 205-025-2), is highly soluble in H_2O , but stable clear solutions only form in the presence of caustic soda in excess
Toxicity data	
LD ₅₀ oral	150 mg/kg rat
subcutaneous	100 mg/kg rat
percutaneous	96 mg/kg rat
intraperitoneal	56 mg/kg rat
LC ₅₀ inhalative	355 mg/m ³ for rats
Suspicion has aroused that PCP is carcinogenic.	
PCP is irritant to skin, mucosa and eyes, however, does not cause sensitization.	
0.001 mg/m ³ is recommended as an occupational exposure limit, because of the suspicion that PCP is carcinogenic.	
Ecotoxicity:	
LC ₅₀ for fish	0.2 mg/l
Activated sludge organisms tolerate	15 mg/l.

Biodegradation of PCP occurs extremely slowly (see Figure 15).

Antimicrobial effectiveness/applications

PCP, in the past one of the most important microbicides for the protection of materials, has been for many years under discussion because of its toxicity, especially its dangerous percutaneous effects, its ecotoxicity and its persistence. Today the pressure for substitution becomes effective and the application of PCP is really on the decline. Considerable quantities are still used for wood protection only, in particular for the protection of freshly cut and sawn timber against coloration through mould growth.

The minimum inhibition concentrations of PCP listed in Table 55 demonstrate its extraordinary broad and equalized activity spectrum. Moreover PCP exhibits strong algicidal effectiveness in concentrations of 2.5–5 mg/l. It is indeed more than a simple microbicide, namely a biocide that is also highly toxic for plants, fish, molluscs, insects and mammals.

Microbicide group (substance class)

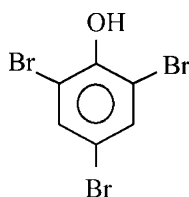
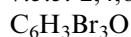
Chemical name

Chemical name

Structural formula

7.5. HALOGENATED PHENOLS

7.5.5. 2,4,6-Tribromophenol



Molecular mass

331.40

CAS-No.

118-79-6

EC-No.

204-278-6

EPA TSCATS

Data Base, Jan. 2001

Synonym/common name

1-hydroxy-2,4,6-tribromobenzene

Supplier

FLUKA CHEMIE

Chemical and physical properties

Appearance

pinkish to white flakes with mild phenolic odour

Content (%)

≥98

Boiling point/range °C (99, 5 kPa)

282–290 (sublimes)

Melting point °C

92–94

Stability

decomposes upon excessive heating

Solubility g/l (20°C)

720 in benzene, 80 in heptane, 0.07 in H₂O at 15°C

Toxicity data

LD₅₀ oral

2 000 mg/kg rat

LC₅₀ inhalative> 1 630 mg/m³ (4 h) for rats

Irritant to skin, mucosa and eyes.

Antimicrobial effectiveness/applications

Tribromophenol is comparable in activity to chlorinated phenols and exhibits considerable insecticidal effectiveness. It is worthwhile mentioning that it is more active against *Cephaloscyus fragrans* than chlorinated phenols. Because of these properties and its relatively favourable toxicity data (as far as they have been established) tribromophenol and its sodium salt are used in wood preservatives to control fungi and insects. Standard application methods of pressure and vacuum impregnation, dipping, soaking, brushing and spraying are suitable.

Table 74 Minimum inhibition concentrations (MIC) of 2,4,6-tribromo-phenol sodium salt in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	25
<i>Chaetomium globosum</i>	50
<i>Penicillium citrinum</i>	50
<i>Polyporus versicolor</i>	35
<i>Polystictus versicolor</i>	70
<i>Rhizopus nigricans</i>	500
<i>Trichoderma viride</i>	250
<i>Tyromyces palustris</i>	100

Microbicide group (substance class)

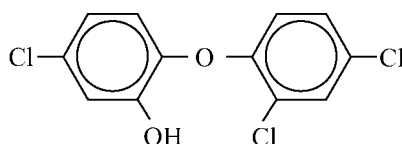
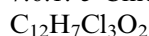
Chemical name

Chemical formula

Structural formula

7.6. PHENOXYPHENOLS

7.6.1. 5-Chloro-2-(2,4-dichlorophenoxy) phenol



Molecular mass	289.55
CAS-No.	3380-34-5
EC-No.	222-182-2; EEC-no.25
EPA-FIFRA	a.i. in antimicrobial pesticide products
Synonym/common name	2,4,4'-trichloro-2'-hydroxydiphenyl ether, Triclosan
Supplier	CIBA SPECIALITY CHEMICALS
Chemical and physical properties	
Appearance	whitish, fine crystalline powder with a slight, faintly aromatic odour
Content %	100
Melting point °C	56–58
Density g/ml	1.55–1.61
Vapour pressure hPa (20°C)	5.32×10^{-6} (2.66×10^{-2} at 100°C)
Flash point °C	223
Auto ignition temperature °C	> 350
pKa value	8.1
Log POW	4.8
Stability	thermal decomposition at 280°C; moderate volatility with water steam, remarkable hydrolytic stability in acid and alkaline solutions at reflux temperatures, sensitive to intense UV-light radiation in solutions; solutions of Triclosan are not stable to chlorine and have only moderate stability in the presence of oxidizing agents
Solubility g/l (20°C)	0.01 in H ₂ O; > 1000 in 95% ethanol, isopropanol, propylene glycol, ethyl acetate, dioctyl phthalate, approx. 1000 in trichloroethylene, 371 in 1 N sodium hydroxide, 4.0 in 1 N sodium carbonate
Toxicity data (source: CIBA)	
LD ₅₀ oral	> 5000 mg/kg rat
dermal	> 6000 mg/kg rabbit
Further toxicological investigations – Summary of results	
Not toxic, not irritating to skin and eyes, when used in formulations. – Not sensitizing.	
Not carcinogenic. Not mutagenic. Not toxic to reproduction. Not teratogenic. No accumulation in organs or tissues; complete elimination.	
Ecotoxicity:	
Tests with activated sludge (batch and continuous tests) at environmentally relevant Triclosan concentrations demonstrate nearly complete removal of the microbicide from waste water, largely as a result of biodegradation.	
LC ₅₀ for bacteria	20 mg/l (3 h)
LC ₀ for Zebra fish	0.5 mg/l
LC ₅₀	0.7 mg/l (48 h)
EC ₅₀ for Daphnia magna	0.4 mg/l (48 h)
EC ₅₀ for algae	0.2 mg/l (72 h)

Antimicrobial effectiveness/applications

Investigations of Regös & Hitz (1974) on the mode of action of Triclosan have shown that at bacteriostatic concentrations the uptake of e.g. essential amino acids by the cytoplasmic membrane is inhibited, whereas bactericidal concentrations of Triclosan cause disorganisation of the cytoplasmic membrane and leakage of low molecular weight cellular components. The minimum inhibition concentrations (MIC; source: CIBA) for Gram-positive bacteria move between 0.01 ppm for species of *Staphylococcus aureus* and 33.0 ppm for lactobacilli. The proliferations of Gram-negative bacteria is inhibited by 0.1 ppm (*Escherichia coli*) to > 1000 ppm Triclosan (*Pseudomonas aeruginosa*). Approximately 3–30 ppm Triclosan are required to suppress the growth of molds and yeasts.

The broad activity spectrum of Triclosan, its stability, its good skin compatibility and favourable toxicological profile make the microbicide to an ideal active ingredient for skin care and oral care products, for hand surface disinfectants, for household products and for the antimicrobial treatment of polymers.

In the EC list of preservatives for cosmetics Triclosan is listed with a maximum authorized concentration of 0.3%. It also has been included into the Japanese positive list for cosmetic ingredients. Percentage of use in US cosmetic formulations: 0.9%.

Microbicide group (substance class)

Chemical name

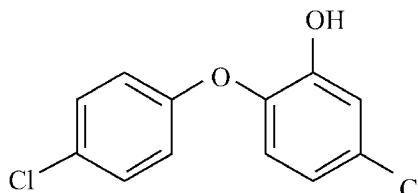
Chemical formula

Structural formula

7.6. PHENOXYPHENOLS

7.6.2. 5-Chloro-2-(4-chlorophenoxy) phenol

$C_{12}H_8Cl_2O_2$



Molecular mass

255.11

CAS-No.

3380-30-1

EC Notification-no.

[N 223]A = acceptable

Synonym/common name

4,4'-dichloro-2-hydroxydiphenyl ether, Tinosan

Supplier

CIBA SPECIALTY CHEMICALS

Chemical and physical properties

Appearance of a solution in
1,2-propylene glycol (1.14.)

yellow-brown liquid with a weak characteristic odour

Content (%)

> 25– < 35

Density g/ml (25°C)

1.07–1.17

Viscosity mPas (25°C)

< 250

Flash point °C

99

Ignition temperature °C

420

Stability

stable up to 150°C; dilution with water results in an insoluble precipitation of the active material

Solubility in % (w/w)

> 50 in alcohols, glycols, glycerine, ethylene glycol n-butyl ether, 24 in mineral oil; can be solubilized in concentrated surfactants

Toxicity data of the active ingredient

(source: CIBA)

LD₅₀ oral

> 2000 mg/kg rat

LD₅₀ dermal

> 2000 mg/kg rat

In tests with rabbits: not irritant to the skin, however, serious eye damage was observed. The a.i. is not sensitizing (Guinea pig test).

Mutagenicity: Ames test: negative; Chromosome aberration test (in vitro): positive;

Micronucleus test (in vivo): negative.

Ecotoxicity:

EC₅₀ for bacteria

~25 mg/l (3 h) (OECD 209)

LC₅₀ for Zebra fish

~2 mg/l (96 h) (OECD 203)

EC₅₀ for *Daphnia magna*

0.32 mg/l (48 h) (OECD 202)

NOEC

0.22 mg/l

EC₅₀ for algae

~0.07 mg/l (OECD 201)

Antimicrobial effectiveness/applications

The MIC in Table 75 give an account of the high effectiveness of Tinosan against a broad spectrum of pathogenic bacteria; however, it has to be mentioned that there is a gap in the activity spectrum with regard to *Pseudomonas aeruginosa*.

As a broad spectrum microbicide with long lasting activity Tinosan is used in home and fabric care products such as dishwashing liquids, laundry detergents, fabric softeners, surface cleaner. Addition rates move between 0.3–0.6%. The incorporation of the pumpable formulation is easy. The persistent activity after application of Tinosan containing household products has been confirmed and reported by Ochs et al. (1999).

Table 75 Minimum inhibition concentrations (MIC) of 5-chloro-2-(4-chlorophenoxy)phenol (> 25–< 35%) in nutrient agar (Source: CIBA)

Test organism	MIC (mg/l)
<i>Gram-positive bacteria</i>	
<i>Corynebacterium xerosis</i> ATCC 373	20.0
<i>Enterococcus hirae</i> ATCC 10541	25.0
<i>Enterococcus faecalis</i> ATCC 51299 (vancomycin-resistant)	50.0
<i>Staphylococcus aureus</i> ATCC 9144	0.2
<i>Staphylococcus aureus</i> ATCC 25923	0.1
<i>Staphylococcus aureus</i> NCTC 11940 (methicillin – resistant)	0.1
<i>Staphylococcus aureus</i> NCTC 12232 (methicillin-resistant)	0.1
<i>Staphylococcus aureus</i> NCTC 10703 (rifampicin-resistant)	0.1
<i>Staphylococcus epidermidis</i> ATCC 12228	0.2
<i>Gram-negative bacteria</i>	
<i>Escherichia coli</i> NCTC 8196	0.07
<i>Escherichia coli</i> ATCC 8739	2.0
<i>Escherichia coli</i> O156 (EHEC)	1.5
<i>Enterobacter cloacae</i> ATCC 13047	1.0
<i>Enterobacter gergoviae</i> ATCC 33028	20
<i>Klebsiella oxytoca</i> DSM 30106	2.5
<i>Klebsiella pneumoniae</i> ATCC 4352	0.07
<i>Listeria monocytogenes</i> DSM 20600	12.5
<i>Proteus mirabilis</i> ATCC 14153	2.5
<i>Proteus vulgaris</i> ATCC 13315	0.2
<i>Pseudomonas aeruginosa</i> ATCC 15442	> 1000
<i>Salmonella choleraesuis</i> ATCC 9184	0.25
<i>Yersinia enterocolitica</i> DSM 4780	25
<i>Fungi</i>	
<i>Aspergillus niger</i> ATCC 6275	150
<i>Candida albicans</i> ATCC 10259	30

7.7. Bisphenols

Bisphenols are composed of two phenolic group which are not separated or separated by various linkages. Some of them exhibit considerable antimicrobial activity, namely those with phenolic groups separated by –CH₂– or –S– or –O–; bisphenols with the OH group at the 2,2'-position to the linkage are the most effective ones. Increasing efficacy is observed with an increasing degree of halogenation which is accompanied by an increase in toxicity.

Microbicide group (substance class)

Chemical name

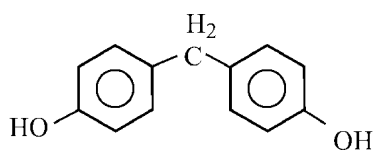
Chemical formula

Structural formula

7.7. BISPHENOLS

7.7.1. Bis-(4-hydroxyphenyl)-methane

C₁₃H₁₂O₂



Molecular mass

200.24

CAS-No.

620-92-8

EC-No.

210-658-2

EPA TSCATS

Data Base, Jan. 2001

Synonym/common name

4,4'-dihydroxydiphenylmethane, 4,4'-methylenebisphenol

Supplier

FLUKA CHEMIE

Chemical and physical properties

Appearance

white powder with phenolic odour

Content (%)

> 98

Melting point °C

162–164

Stability

stable under normal conditions

Solubility g/l

practically insoluble in H₂O; soluble in NaOH and organic solvents

Toxicity data

LD₅₀ oral 4950 mg/kg rat
 Irritates skin, mucous membranes and eyes.
 Ecotoxicity data are not yet available.

Antimicrobial effectiveness/applications

Due to its moderate antimicrobial activity the compound has not gained importance as a microbicide in practice.

Table 76 Minimum inhibition concentrations (MIC) of bis-(4-hydroxyphenyl)-methane in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	500
<i>Staphylococcus aureus</i>	500
<i>Aspergillus niger</i>	> 1000
<i>Chaetomium globosum</i>	500
<i>Penicillium glaucum</i>	750

Microbicide group (substance class)

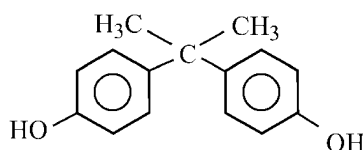
Chemical name

Chemical formula

Structural formula

7.7. BISPHENOLS

7.7.2. 2,2-Bis(4-hydroxyphenyl) propane

C₁₅H₁₆O₂

Molecular mass

228.29

CAS-No.

80-05-7

EC-No.

201-245-8

EPA TSCATS

Data Base, Jan. 2001

Synonym/common name

4,4'-isopropylidenediphenol,
 4,4'-dihydroxydiphenyldimethylmethane, Bisphenol A
 FLUKA CHEMIE

Supplier

Chemical and physical properties

Appearance

white crystalline powder with phenolic odour

Content (%)

~97

Boiling point/range °C (0.13 kPa)

190

Melting point °C

153–156

Stability

stable under normal conditions

Solubility

virtually insoluble in H₂O; soluble in aqueous solution of
 caustic soda, in ethanol, acetone

Toxicity data

LD₅₀ oral

2400 mg/kg mouse

3250 mg/kg rat

4000 mg/kg guinea pig

LD₅₀ intraperitoneal

150 mg/kg mouse

Mildly irritant to the skin, however, severely irritant to the eyes.

Ecotoxicity data are not yet available.

Antimicrobial effectiveness/applications

The condensation of phenol (7.1.) with 1 mol acetone leads to the Bisphenol A which has little interest as a microbicide. Its antifungal activity is not very distinctive; the antibacterial efficacy is not worth a mention.

The antioxidizing properties of Bisphenol A are of use for plasticisers.

Microbicide group (substance class)

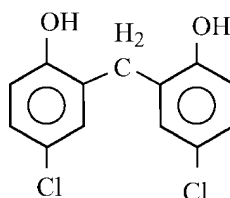
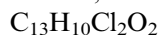
Chemical name

Chemical formula

Structural formula

7.7. BISPHENOLS

7.7.3. 2,2'-Methylenebis (4-chlorophenol)



Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

269.14

97-23-4

202-567-1

Data Base/Jan. 2001

bis(5-chloro-2-hydroxyphenyl) methane, Dichlorophen

BDH, UK; FLUKA CHEMIE

Chemical and physical properties

Appearance

white crystalline powder, sometimes with phenolic odour caused by traces of 4-chlorophenol (7.5.1.), the starting product which is converted acid-catalytically with formaldehyde (2.1a.) to Dichlorophen

Content (%)

> 98

Melting point °C

176

Density g/ml (20°C)

1.5

Vapour pressure hPa (100°C)

< 0.01

pKa values

8.7/12.6

Stability

stable under normal conditions, not volatile with water steam

Solubility g/l (20°C)

0.07 in H₂O (0.2 at 50°C), 500 in 10% NaOH, 400 in butanol, 12 in toluene, highly soluble in ethanol

Toxicity data

LD₅₀ oral

1506 mg/kg rat

1000 mg/kg mouse

1250 mg/kg guinea pig

17 mg/kg rat

LD₅₀ intravenous

Skin irritation (rabbit test): mild; 500 mg/24 h.

Eye irritation (rabbit test): severe; 5×10^{-2} mg/24 h.

No significant sensitization potential; photosensitization is possible.

Dichlorophen is neither mutagenic nor teratogenic.

Ecotoxicity:

LC₀ for *Leuciscus idus*

0.5 mg/l (72 h)

LC₁₀₀

1 mg/l

Biodegradation proceeds slowly (see Figure15).

Concentrations up to 20 mg/l have no adverse effect on the degradation potential of activated sludge organisms.

Antimicrobial effectiveness/applications

The minimum inhibition concentrations for Dichlorophen in Table 55 enlighten the activity spectrum of the compound; obviously there is a gap for Pseudomonads. The efficacy against yeasts and fungi is, however, strong and equalized. The optimal pH for bactericidal activity is 5–6. At that pH *Staphylococcus aureus* is killed totally at 20°C within 10 min through 250 mg Dichlorophen/litre. However, in the presence of serum the bactericidal concentration of Dichlorophen is much higher (> 1000 mg/litre). There is a big discrepancy between bactericidal and bacteriostatic activity; the latter is especially distinctive at pH 8. With regard to its application as a microbicide for the protection of materials Dichlorophen offers a lot of advantageous properties: stable, not volatile, not leachable, soluble in oil and alkaline solutions, good skin compatibility. It is used as an active ingredient in slimicides, as a preservative for lubricoolants, or the fungicidal treatment of textiles, paper, cardboard, adhesives. Dichlorophen of high purity (practically odourless) may also be used in cosmetics, e.g. deodorants, antiseptic soaps. But there is risk of coloration for Dichlorophen-containing soaps at exposure to light, especially at pH > 7.

Both sodium salts of Dichlorophen are available for practical applications: monosodium Dichlorophen (CAS-No. 10187-52-7; EC-No. 233-457-1) and disodium Dichlorophen (CAS-No. 22232-25-3; EC-No. 244-853-9). In the textile industry there is a special interest in the application of fatty acid esters of Dichlorophen (9.10).

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

pKa values

Stability

Solubility g/l (20°C)

Toxicity data

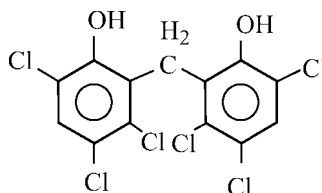
LD₅₀ oral for children
for adultsLD₅₀ oral
dermal
subcutane
intraperitoneal
intravenousLC₅₀ inhalative

Irritant to the skin and mucous membranes – absorption through the skin.

Neurotoxic, embryotoxic, tumorigenic.

7.7. BISPHENOLS

7.7.4. 2,2'-Methylenebis (3,4,6-trichlorophenol)

C₁₉H₆Cl₆O₂

406.92

70-30-4

200-733-8

Data Base, Jan. 2001

bis(3,5,6-trichloro-2-hydroxyphenyl)methane,
Hexachlorophen
FLUKA CHEMIEwhite crystalline powder which results from the
condensation of 2,4,5-trichlorophenol (7.5.3.) with
formaldehyde in presence of concentrated sulphuric acid
≥98

164–165

5.4/10.9

stable under normal conditions

1010 in acetone, 500 in ethanol, 56 in toluene; virtually
insoluble in H₂O; soluble in alkaline solutions (forms
alkali salts)

250 mg/kg body weight

350 mg/kg body weight

56 mg/kg rat

1840 mg/kg rat

7.65 mg/kg rat

22 mg/kg rat

7.5 mg/kg rat

340 mg/m³ for rats

Antimicrobial effectiveness/applications

Hexachlorophen is highly effective against bacteria, especially against Staphylococci, not as effective against fungi.

Minimum inhibition concentrations for

Staphylococcus aureus	approx. 1 mg/l
Pseudomonas aeruginosa	approx. 500 mg/l
Fungi	500–1000 mg/l

Optimum pH for bacteriostatic activity is 8. A maximum of bactericidal action is achieved at pH 5–6. Under these conditions Staphylococcus aureus is killed within 5 min by 1000 mg Hexachlorophen/l.

Hexachlorophen has been used as a microbicide in cosmetics, medical soaps and detergents, and in textiles with antimicrobial effectiveness. However, because of its toxicity, especially its neurotoxicity and the potential absorption through the skin, the application has been banned worldwide.

Microbicide group (substance class)

Chemical name

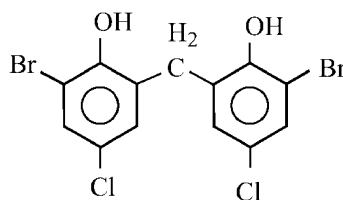
Chemical formula

Structural formula

7.7. BISPHENOLS

7.7.5. 2,2'-Methylenebis(6-bromo-4-chlorophenol)

$C_{13}H_8Br_2Cl_2O_2$



Molecular mass

426.92

CAS-No.

14435-29-7

EC-No.

unknown; EEC-no. 37

Synonym/common name

3,3'-dibromo-5,5'-dichloro-2,2'-
dihydroxydiphenylmethane, Bromochlorophen
MERCK INC.

Supplier

Chemical and physical properties

Appearance

white crystalline powder with a slight phenolic odour

Content (%)

~99

Melting point °C

188–191

Stability

not stable under light; incompatible (inactivation) with
non-ionic and cationic surfactants and proteins

Solubility g/l (20°C)

95 in ethanol, 70 in n-propanol, 40 in isopropanol, 25 in
1,2,-propyleneglycol, 5 in paraffin oil, <1 in glycerine,
<1 in H₂O

Toxicity data

LD₅₀ oral

3700–8153 mg/kg rat

dermal

> 10000 mg/kg rat

intraperitoneal

580 mg/kg rat

LC₅₀ inhalative

800 mg/m³ (5 h) for rats

Good skin compatibility, only moderately irritant to mucous membranes, no sensitization (guinea pig test).
Photosensitization has not been observed.

Not mutagenic, not teratogenic.

Antimicrobial effectiveness/applications

Bromochlorophen is produced by bromination of 2,2'-methylenebis(4-chlorophenol) (7.7.3.). The minimum inhibition concentrations range between 10 mg/litre against Staphylococcus aureus and 1000 mg/litre against Escherichia coli and Pseudomonas aeruginosa. The efficacy against fungi is not very distinctive. Mainly because of its good skin compatibility Bromochlorophen is used as a preservative in cosmetics and an additive in deodorants. Optimum pH range 5–6. The EC positive list of preservatives for cosmetics mentions a maximum authorized concentration of 0.1%.

Microbicide group (substance class)

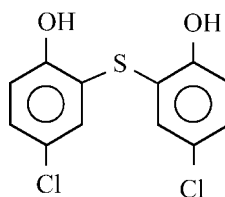
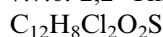
Chemical name

Chemical formula

Structural formula

7.7. BISPHENOLS

7.7.6. 2,2'-Thiobis(4-chlorophenol)



Molecular mass

287.18

CAS-No.

97-24-5

EC-No.

202-568-7

Synonym/common name

2,2'-dihydroxy-5,5' dichloro-diphenylsulphide, Fentichlor

Supplier

COCKER CHEM. CO.

Chemical and physical properties

Appearance

white powder resulting from the reaction of 4-chlorophenol (7.5.1.) with sulphur dichloride in the presence of AlCl_3

Content (%)

~98

Melting point °C

174

Stability

solutions exposed to light change colour to brown

Solubility g/l

0.03 in H_2O ; readily soluble in alkaline solutions and organic solvents; the sodium salt is available as 50% solution

Toxicity data

LD₅₀ oral

3250 mg/kg rat

Can cause photosensitization.

Antimicrobial effectiveness/applications

The similarity of Fentichlor to other bisphenols is demonstrated by the lack in activity against *Pseudomonads*. Remarkable is the broad and equalized spectrum of effectiveness against fungi and yeasts and even algae.

Low water solubility, tendency to coloration, lack in effectiveness against *Pseudomonads* have limited the applications of Fentichlor as a microbicide for material protection. It can be used as a preservative in lubricoolants to overcome problems with fungal growth, especially in so-called 'bioresistant' lubricoolants which inhibit the growth of bacteria but not that of fungi. The 50% sodium salt solution of Fentichlor has been applied as a slimicide and algicide.

Table 77 Minimum inhibition concentrations (MIC) of Fentichlor in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	75
<i>Pseudomonas aeruginosa</i>	3500
<i>Staphylococcus aureus</i>	35
<i>Aspergillus niger</i>	50
<i>Aureobasidium pullulans</i>	< 20
<i>Chaetomium globosum</i>	75
<i>Cladosporium herbarum</i>	35
<i>Cladosporium sphaerospermum</i>	50
<i>Coniophora cerebella</i>	< 20
<i>Penicillium citrinum</i>	20
<i>Penicillium digitatum</i>	< 20
<i>Penicillium funiculosum</i>	35
<i>Penicillium glaucum</i>	10
<i>Penicillium italicum</i>	50
<i>Polyporus versicolor</i>	35
<i>Trichophyton pedis</i>	10
<i>Trichoderma viride</i>	50
<i>Candida albicans</i>	50
<i>Torula rubra</i>	50
Fresh water algae	20

The mode of action of Fentichlor was studied by Huga and Bloomfield (1971), using *Staphylococcus aureus* and *Escherichia coli* as test organisms. They found that minor concentrations of Fentichlor are reversibly adsorbed and accordingly act bacteriostatically (see Part-One, Chapter 2). The undissociated active agent is taken up by the cell wall and cell membrane, the latter probably being the main site of adsorption and main site of action. In spite of higher affinity of Fentichlor to *E. coli* in comparison to *St. aureus*, the latter is more susceptible to the antibacterial action of Fentichlor. Apparently the lipid-rich nature of the cell walls of *E. coli* act as an absorbing barrier restraining the access of the active compound to its site of action.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility

Toxicity data

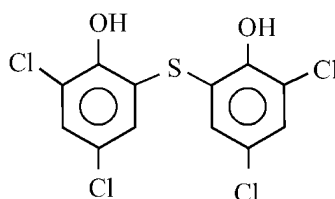
LD₅₀ oral

Bithionol may act as a photosensitizing substance.

7.7. BISPHENOLS

7.7.7. 2,2'-Thiobis(4,6-dichlorophenol)

C₁₂H₆Cl₄O₂S



356.01

97-18-7

202-565-0

2,2'-dihydroxy-3,3',5,5'-tetrachlorodiphenylsulphide,
Bithionol

COCKER CHEM. CO.

white crystalline powder resulting from the reaction of 2,4-dichlorophenol with sulphur dichloride in the presence of AlCl₃

~98

188

solutions, primarily alkaline solutions change colour to brown

virtually insoluble in H₂O, readily soluble in ethanol, diethyl ether, acetone, glacial acetic acid and dilute alkalis

1430 mg/kg rat

2100 mg/kg mouse

Antimicrobial effectiveness/applications

Due to its higher degree of chlorination Bithionol shows in comparison to Fentichlor even more activity against gram-positive bacteria, but has the same gap for Pseudomonads in its activity spectrum. There is also for bisphenols a typical discrepancy between microbistatic and microbicidal concentrations, differing from 1 to 1000 mg/litre, e.g. for *Staphylococcus aureus*.

With regard to the application of Bithionol as a microbicide for material protection what is said for Fentichlor (7.7.6.) is valid for Bithionol, too.

7.8. Nitrophenols

The introduction of the nitro group into the phenol molecule leads to an increase in acidity and antimicrobial effectiveness, the latter being superior to most of the halophenols. However, the gain in activity is accompanied by an equal gain in toxicity. The yellow colour of nitrophenols is an additional handicap in the application of the compounds as microbicides for the protection of materials. In the meantime nitrophenols may be marked as old-line microbicides which are no longer of practical importance.

Chemical name	7.8.1. 4-Nitrophenol
Chemical formula	$C_6H_5NO_3$
Structural formula	
Molecular mass	139.11
CAS-No.	100-02-7
EC-No.	202-811-7
EPA TSCATS	Data Base, Jan. 2001
Synonym/common name	p-nitrophenol, 4-hydroxynitrobenzene
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	yellow crystals
Content (%)	> 99.5
Boiling point/range °C (101 kPa)	279
Melting point °C	112–115
Dissociation constant	1.38×10^{-9}
Stability	stable under normal conditions; in caustic soda solution 4-nitrophenol sodium salt is formed which may be isolated as the dihydrate (mp > 300°C; CAS-No. 824-78-2, EC-No. 212-536-4)
Solubility	4-nitrophenol is sparingly soluble in H_2O , but readily soluble in organic solvents and alkaline solutions
Toxicity data	
LD ₅₀ oral	202 mg/kg rat
dermal	1024 mg/kg rat
LD ₅₀ oral	282 mg/kg mouse
intraperitoneal	75 mg/kg mouse
Irritant to skin, mucosa and eyes.	
EPA Genetox Program 1988: Inconclusive.	

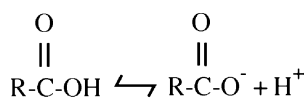
Antimicrobial effectiveness/applications

4-Nitrophenol is a broad spectrum microbicide, but out of use nowadays because of its toxicity and colouration effects. It was mainly used as a preservative in the leather industry.

8. Acids

Among the large number of organic and inorganic acids there are only a few which are used as preservatives, especially in the food and cosmetic industry. In this section only those acids are listed which really exhibit antimicrobial activity; peroxy-carboxylic acids are described under 21. Oxidizing agents. That means, acids which act as acidulants, buffers, flavouring agents or synergists to antioxidants are not discussed here, e.g. phosphoric acid, citric acid, malic acid, adipic acid, succinic acid, glutaric acid, ascorbic acid, isoascorbic acid. Acids in general may be helpful in the combat against microbes, as low pH values facilitate the destruction of microbes, e.g. by heat, with the advantage that one needs shorter sterilization or pasteurization processing times thus saving energy and preserving product quality. In addition at low pH values bacterial growth generally is suppressed and spore germination is delayed or prevented. Finally acidulants lowering the pH of, for example, foods support the effect of antimicrobial active acids, as these are only effective in acidic preparations.

The microbicidal acids belong to the membrane-active substances. Typical is that acids display significant antimicrobial activity only when they are present in their undissociated state. A carboxylic acid for instance dissociates according to the following equation.



Undiss. state

Diss. state

Table 78 *pKa* values of acid compounds used as microbicides

Acidic microbicide	<i>pKa</i>
Formic acid	3.8
Acetic acid	4.8
Propionic acid	4.9
Lactic acid	3.8
Dehydroacetic acid	5.4
Sorbic acid	4.8
Benzoic acid	4.2
Salicylic acid	3.0
Methyl <i>p</i> -hydroxybenzoate	8.5
Propyl <i>p</i> -hydroxybenzoate	8.1
2-Phenyl-phenol	11.6
4-Chloro-3-methyl-phenol	9.6
Sulphurous acid	1.8/6.9
Boric acid	9.1

The acidity is characterized by the dissociation constant *K*; the greater is *K*, the higher is the concentration of H^+ ions or the stronger is the acid. With regard to the utility of acids as microbicides it is, however, as already said, important to have knowledge about the availability of the undissociated form. That is characterized by the pK_a value indicating the pH value at which 50% of the acid is present in the undissociated state. pK_a values of a selection of microbicides having acid character are listed in Table 78.

The antimicrobial active acids' mode of action is based on the ability of the undissociated forms to interact with or to pass through the membrane of the microbial cell which normally is negatively charged thus being a barrier for the negatively charged forms of the acids. In their undissociated state the acids may alter the membrane permeability of the microbial cell and interfere with many enzymatic processes in the cell. That means nutrient transport inhibition is mainly responsible for the antimicrobial effect of acids.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.1. Formic acid
Chemical formula	CH_2O_2
Structural formula	$ \begin{array}{c} H-C=O \\ \\ OH \end{array} $
Molecular mass	46.03
CAS-No.	64-18-6
EC-No.	200-579-1; EEC-no. 14
EPA TSCATS	Data Base, Jan. 2001
Synonym/common name	formylic acid, hydrogencarboxylic acid, methanoic acid
Supplier	BASF, HOUGHTON CHEM. CORP., HUELS, MERCK, RdH
Chemical and physical properties	
Appearance	colourless fluid with a pungent odour
Content (%)	~98
Boiling point/range °C (101 kPa)	100–101
Solidification point °C	8.5
Density g/ml (20°C)	1.22
Vapour pressure hPa (20°C)	59.6
Refractive index nD (20°C)	1.371
Flash point °C	54.44
Auto ignition temperature °C	601
Upper flammability limit % v/v i. air	57
Lower flammability limit % v/v. i. air	18
Dissociation constant	1.77×10^{-4}
pK_a value	3.8

Stability	among the organic acids is formic acid a special one, as it can react not only as an acid but also as an aldehyde (see structural formula) with reducing properties
Solubility	miscible with water, alcohol, glycerine, ether
Toxicity data	
LD ₅₀ oral	1100 mg/kg rat
	700 mg/kg mouse
intraperitoneal	940 mg/kg mouse
Intravenous	145 mg/kg mouse
LC ₅₀ on inhalation	15 g/m ³ (15 min) for rats
	6.2 g/m ³ (15 min) for mice
Concentrated formic acid extremely irritates skin, mucosa and eyes.	
Carcinogenic and teratogenic effects are not observed.	
Acceptable daily intake (ADI value): 3 mg/kg body weight.	
Occupational exposure limits: 9(5) mg/m ³ (ppm).	

Antimicrobial effectiveness/applications

Formic acid exhibits its antimicrobial activity primarily in the undissociated state, i.e. at pH below 3.5 and therefore partly as an acidulant. The efficacy covers especially yeasts and some bacteria; lactic acid bacteria and moulds are relatively resistant. Ethyl formate (9.1.), a generator of formic acid may also be applied for preservation purposes. Sodium formate (CAS-no. 141-53-7, EC-no. 205-488-0) and potassium formate (CAS-no. 590-29-4, EC-no. 209-677-9) – both are crystalline white powders which are highly soluble in water- are used for the preservation of acid foods (addition rates: 0.1–0.4%), frequently in combination with benzoic acid (8.1.9.) or sorbic acid (8.1.5.). In the EC list of preservatives permitted for the incorporation into cosmetic products formic acid and its sodium salt are mentioned with a maximum authorized concentration of 0.5% (acid).

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.2. Acetic acid
Chemical formula	C ₂ H ₄ O ₂
Structural formula	$\begin{array}{c} \text{H}_3\text{C}-\text{C}=\text{O} \\ \\ \text{OH} \end{array}$
Molecular mass	60.05
CAS-No.	64-19-7
EC-No.	200-580-7
EPA TSCATS	Data Base, Jan. 2001
Synonym/common name	ethanoic acid, methanecarboxylic acid
Supplier	DOW, HOECHST, HOUGHTON CHEM. CORP., HUELS, MERCK, RdH
Chemical and physical properties	
Appearance	colourless fluid with a characteristic pungent odour and lachrymal effect
Content (%)	> 99
Boiling point/range °C (101 kPa)	117–118
Melting point °C	16.5
Density g/ml (20°C)	1.049
Vapour pressure hPa (20°C)	15
Refractive index n _D (25°C)	1.3698
Flash point °C	40
Upper flammability limit % v/v i. air	19.9
Lower flammability limit % v/v i. air	4
pK _a value	4.8

Stability	stable under normal conditions, hygroscopic
Solubility	miscible with water, alcohol, ether, chloroform, glycerine and other organic solvents, insoluble in carbon disulphide
Toxicity data	
LD ₅₀ oral	3310 mg/kg rat
intravenous	525 mg/kg mouse
dermal	1.06 ml/kg rabbit
LC ₅₀ on inhalation	5620 ml/m ³ (1 h) for mice
Severely irritant to the skin, mucosa and eyes.	
ADI value	15 mg/kg body weight
Occupational exposure limits	25 (10) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

At lower pH values (< 4.5) acetic acid inhibits the growth of bacteria, yeasts and to a lesser extent the growth of moulds. The inhibitory effect is greater than due to pH alone. One assumes that undissociated acetic acid because of its good lipid solubility can penetrate the microbial cell and exert its antimicrobial effect. Bacteria of the genus *Acetobacter* can produce acetic acid by the oxidation of alcohol. In combination with heat acetic acid is especially effective. Vinegar is one of the oldest preservatives and flavouring agents for food and also today acetic acid's application as a preservative is in food only. An acetic acid containing salt is sodium diacetate = sodium acetate + 1 mol acetic acid (CH₃-COONa-CH₃-COOH; M = 142.09; CAS-no. 126-96-5), a white hygroscopic powder (decomposition at 150°C) which is highly soluble in water (~1 g/l ml), where it releases acetic acid. pH of a 1:10 solution in H₂O approx. 4.5–5.0. As a permitted food preservative sodium diacetate is used as a mould inhibitor in the making bread process; addition rate: up to 0.3%.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.3 Propionic acid
Chemical formula	C ₃ H ₆ O ₂
Structural formula	H ₃ C-CH ₂ -COOH
Molecular mass	74.08
CAS-No.	79-09-4
EC-No.	201-176-3; EEC-no. 2
Registration	EC: permitted as food additive (E 280) USA, FDA: considered as GRAS
EPA TSCATS	Data Base, Jan., 2001
Synonym/common name	ethanecarboxylic acid, ethylformic acid, methylacetic acid
Supplier	BASF, BP CHEMICALS, CELANESE, DOW-UNION CARBIDE, DSM, EASTMAN, TOYO GOSEI
Chemical and physical properties	
Appearance	clear, colourless liquid with a strong pungent, rancid odour
Content (%)	> 99
Boiling point/range °C (101 kPa)	141
Solidification point °C	–21
Density g/ml (20°C)	0.993
Vapour pressure hPa (20°C)	13.3
Refractive index nD (20°C)	1.386
Flash point °C	52
Auto ignition temperature °C	485
Upper flammability limit % v/v i.air	12.1
Lower flammability limit % v/v i.air	2.9
pKa value	4.9
Stability	stable under normal conditions
Solubility	miscible with water, highly soluble in organic solvents

Toxicity data

LD ₅₀ oral	2600 mg/kg rat
parenteral	3500 mg/kg rat
intravenous	625 mg/kg mouse

Propionic acid and its salts (sodium propionate and calcium propionate) are severely irritant to skin, mucosa and eyes.

ADI value:	10 mg/kg body weight.
Occupational exposure limits	30 (10) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

Propionic acid is considered as benzoic acid (8.1.9) and sorbic acid (8.1.5.) a lipophilic acid which is an active microbicide in the undissociated form only; in consequence it exhibits optimum efficacy at pH values below 5. Propionic acid is primarily effective against moulds. The activity against yeasts is minimal and the activity against bacteria is poor. Microbes belonging to the genus *Propionibacterium* produce propionic acid during fermentation of Swiss Gruyère cheese. However, *Bacterium mesentericus* is an exception as it is very sensitive to the effect of propionic acid. As a preservative propionic acid is used for the protection of acid media. However, instead of the corrosive acid one preferably applies sodium propionate (CAS-no. 137-40-6; EC-no. 205-290-4; M = 96.1), or calcium propionate (CAS-no. 4075-81-4; EC-no. 223-795-8; M = 186.22). Both salts are free flowable, white, water soluble powders; the water solubility at 100°C amounts to 1500 g sodium propionate/l and 558 g calcium propionate/l. The main field of application for propionates is the baking industry where they can protect bread and cakes from the growth of moulds and the proliferation of *Bacterium mesentericus* which causes rope in bread. Addition rates: 0.15–0.3%. Propionates are also used for the protective treatment of cheese. In the EC list of preservatives which cosmetic products may contain propionic acid and its salts are listed with a maximum authorized concentration of 2% (acid).

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.4. DL-Lactic acid
Chemical formula	C ₃ H ₆ O ₃
Structural formula	H ₃ C-CH(OH)-COOH
Molecular mass	90.08
CAS-No.	50-21-5
EC-No.	209-954-4
EPA TSCATS	Data Base, Jan. 2001 – FDA considers lactic acid as GRAS
Synonym/common name	racemic lactic acid, 2-hydroxypropionic acid
Supplier	FLUKA CHEMIE, RdH

Chemical and physical properties

Appearance	colourless, odourless, highly viscous and hygroscopic liquid
Content (%)	~98
Boiling point/range °C (101 kPa)	119
Melting point °C	18
Density g/ml (20°C)	1.21
Flash point °C	11.3
pK _a value	3.79
Stability	stable under normal conditions
Solubility	miscible with water, alcohol and glycerine, soluble in ether, insoluble in trichloromethane and benzene

Toxicity data

LD ₅₀ oral	3543 mg/kg rat
	4875 mg/kg mouse
	1810 mg/kg guinea pig
LD ₅₀ dermal	> 2000 mg/kg rabbit

Moderately to severely irritant to skin, mucosa and eyes.

ADI value: FAO has set no limit.

Antimicrobial effectiveness/applications

Lactic acid is a natural constituent of food; it is manufactured through microbial fermentations, e.g. by *Lactobacilli*. Synthetically lactic acid can be produced out of propylene and N_2O_4 . Fermentation processes are the oldest methods for the protection of food. Lactic acid produced in such processes lowers the pH to levels unfavourable for the growth of spoilage organisms, that means it is mainly active as an acidulant. However, efficacy is observed against anaerobic bacteria, although at concentrations higher than 0.5%. Consequently for broader antimicrobial effectiveness lactic acid has to be combined with other microbicides active against yeasts and moulds, such as benzoic acid (8.1.9.) or sorbic acid (8.1.5.). Remarkable is the ability of lactic acid to inhibit specifically mycotoxin formation (Reiss, 1976; Lück, 1980). Ziaudin et al. (1993) demonstrated in *in vitro* studies the growth inhibitory effect of lactic acid on pathogenic bacteria. A combination of lactic acid and NaCl markedly enhanced the inhibitory effect against different species of bacteria responsible for microbial spoilage of meat and meat products as well as those causing meat borne infections and intoxicants.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.5. Sorbic acid
Chemical formula	$C_6H_8O_2$
Structural formula	$H_3C-CH=CH-CH=CH-COOH$
Molecular mass	112.13
CAS-No.	110-44-1
EC-No.	203-768-7; EEC-no. 4
Registrations	EC: permitted food additive (E 200) GRAS status in the USA
Synonym/common name	trans-trans-2,4-hexadienoic acid, 1,3-pentadiene-1-carboxylic acid, 2-propenylacrylic acid
Supplier	CELANESE, CHEMINOVA, NIPPON GOSEI, UENO
Chemical and physical properties	
Appearance	white crystalline powder with a distinctive odour
Content (%)	~100
Boiling point/range °C (101 kPa)	228 (decomposition)
Melting point °C	134
Vapour pressure hPa (20°C)	0.013
Flash point °C	127
pK _a value	4.76
Stability	sensitive to light; in contrary to potassium sorbate and calcium sorbate the sodium salt is extremely sensitive to oxidation
Solubility g/l (20°C)	~2 in H ₂ O, ~120 in ethanol, ~85 in isopropanol, ~5 in propylene glycol, ~3 in glycerine, ~5–10 in oils, ~110 in acetic acid
Toxicity data	
LD ₅₀ oral	7360 mg/kg rat 3200 mg/kg mouse
LD ₅₀ intraperitoneal	800 mg/kg rat 2820 mg/kg mouse
LD ₅₀ subcutane	2820 mg/kg mouse
Irritant to skin, mucosa and eyes.	
ADI value (FAO/WHO): 0-25 mg/kg body weight.	

Antimicrobial effectiveness/applications

Sorbic acid is a naturally occurring compound, its lactone ('sorbic oil') is found in *Sorbus acuparia*. It is justified to characterize sorbic acid as one of the least toxic of all the preservative agents known. In its undissociated form it is a membrane active agent which due to its unsaturated character additionally may exhibit electrophilic activity. Therefore sorbic acid is able to penetrate the microbial cell membrane and to inhibit nutrient transport

and enzymes. In accordance with its pK_a value of 4.76 sorbic acid is most effective at pH 4 or below in its undissociated form; however there is considerable antimicrobial activity of sorbic acid observed also at pH values up to 6.0-6.5. This may be attributed to the ability of sorbic acid to partial intramolecular cyclisation to the delta lactone of 5-hydroxy-2-hexene acid, an electrophilic, neutral substance the activity of which is not that much dependent on pH as is the acid. Hence the breadth of application is for sorbic acid larger than for other lipophilic acid preservatives.

Sorbic acid inhibits especially the growth of moulds and yeasts; its efficacy against bacteria is not that equalized and consequently not reliable. At addition rates between 0.05 and 0.3% sorbic acid is used as a preservative for foodstuffs (especially beverages, including wine), pharmaceuticals and cosmetics. The sodium, potassium and calcium salts are also available; the most frequently used salt form is, however, potassium sorbate (CAS-no. 24634-61-5; EC-no. 246-376-1; E 202), because of its high solubility in water (1390 g/l).

In the EC list of preservatives allowed for the protection of cosmetics sorbic acid is listed with a maximum authorized concentration of 0.6%. -Percentage of use in US cosmetic formulations: 0.89% sorbic acid; 0.39% potassium sorbate.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.6. n-Octanoic acid
Chemical formula	C ₈ H ₁₆ O ₂
Structural formula	CH ₃ (CH ₂) ₆ -COOH
Molecular mass	144.22
CAS-No.	124-07-2
EC-No.	204-677-5
USA	considered as GRAS
Synonym/common name	caprylic acid
Supplier	CRODA Ltd., RdH
Chemical and physical properties	
Appearance	clear, colourless, oily fluid with a faint rancid odour
Content %	~99
Boiling point/range °C (101 kPa)	239
Melting point °C	16
Density g/ml (20°C)	0.909
Refractive index n _D (20°C)	1.4285
pK _a value	4.9
Stability	stable under normal conditions
Solubility g/l (20°C)	0.7 in H ₂ O; freely soluble in ethanol, solvents for fats and glacial acetic acid
Toxicity data	
LD ₅₀ oral	8.19–12.37 g/kg rat
Severely irritant to skin, mucosa and eyes.	

Antimicrobial effectiveness/applications

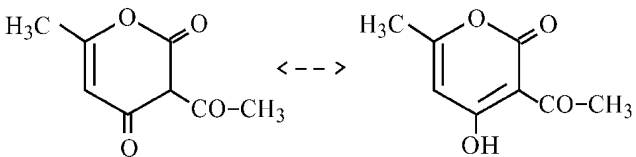
Caprylic acid is effective against bacteria and fungi, optimum pH range: < 6. Due to its limited water solubility it may be insufficiently active to protect the water phase of emulsion systems against microbial proliferation. A main application field for caprylic acid is the incorporation into cheese wrappers as an antimicrobial active ingredient. Caprylic acid may also be used as a flavouring adjuvant (Doores, 1993). Worthy on note is that caprylic acid exhibits insecticidal activity, too.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.7. Undec-10-enoic acid
Chemical formula	C ₁₁ H ₂₀ O ₂
Structural formula	CH ₂ =CH-(CH ₂) ₈ -COOH
Molecular mass	184.28

CAS-No.	112-38-9
EC-No.	203-965-8; EEC-no. 18
EPA/TSCATS	Data base, Jan, 2001
Synonym/common name	undecylenic acid, 10-undecenoic acid
Supplier	RdH
Chemical and physical properties	
Appearance	crystals or colourless liquid with a distinctive odour
Content %	~99
Boiling point/range °C (101 kPa)	275 (decomposition)
Melting point °C	24.5
Density g/ml (25°C)	0.912
Refractive index nD (25°C)	1.4478
Flash point °C	149
Stability	stable under normal conditions, naturally occurring in perspiration; synthetically accessible by pyrolysis of ricinoleic acid, the glyceride which is contained in castor oil;
Solubility	virtually insoluble in H ₂ O, soluble in many organic solvents, e.g. alcohols, ether, trichloromethane
Toxicity data	
LD ₅₀ oral	2500 mg/kg rat 8150 mg/kg mouse
LD ₅₀ intraperitoneal	960 mg/kg mouse
LD ₅₀ dermal	50 mg/kg guinea pig
Irritant to mucous membranes at concentrations > 1%.	

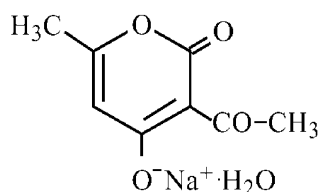
Antimicrobial effectiveness/applications

The antimicrobial activity of undecylenic acid is widely restricted to the inhibition of moulds including pathogenic fungi. The pH optimum is in the acidic range (4.5–6). Nevertheless undecylenic acid is listed among the preservatives allowed for the protection of cosmetics in the EC (maximum authorized concentration: 0.2%). Undecylenic acid may also be applied in dermatics as a fungicide (addition rates 2–10%). For preservation purposes it is recommended to use undecylenic acid only in combination with bactericides to complete the spectrum of effectiveness.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.8. Dehydroacetic acid (DHA)
Chemical formula	C ₈ H ₈ O ₄
Structural formula	 The diagram shows two chemical structures of dehydroacetic acid (DHA) in equilibrium, separated by a double-headed arrow. The structure on the left is 3-acetyl-6-methyl-2,4-pyran-dione, a six-membered ring with an oxygen atom at the top, a carbonyl group at the 2-position, a methyl group at the 6-position, and an acetyl group at the 3-position. The structure on the right is 3-acetyl-4-hydroxy-6-methyl-2-pyrone, which is identical except for the presence of a hydroxyl group at the 4-position instead of a carbonyl group.
Molecular mass	168.14
CAS-No.	520-45-6
EC-No.	212-227-4; EEC-no. 13
USA	classification as GRAS
Synonym/common name	3-acetyl-6-methyl-2[H]-pyran-2,4[3H]-dione resp. 3-acetyl-4-hydroxy-6-methyl-2[H]-pyran-2-one
Supplier	LONZA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless, crystalline, tasteless powder
Content (%)	> 98

Boiling point/range °C (101 kPa)	269.9
Melting point °C	109–101 (sublimation)
pK _a value	5.27
Stability	solutions of DHA tolerate heating up to 120–130°C for 1 h; degradation at pH values > 6; discolouration with iron compounds; incompatible with nonionic surfactants
Solubility g/l	<1 in H ₂ O, 50 in methanol, 30 in ethanol, 17 in propylene glycol, 220 in acetone, 16 in olive oil, 30 in tetrachloromethane, 180 in benzene

Chemical name	8.1.8a. Sodium dehydroacetate monohydrate (DHA-Na)
Chemical formula	C ₈ H ₇ NaO·1H ₂ O
Structural formula	



Molecular mass	208.15
CAS-No.	4418-26-2
EC-No.	224-580-1
Permissions for cosmetics	listed in the EEC cosmetic directive, accepted in Japan and USA
EPA TSCA	chemical inventory
Synonym/common name	sodium 1-(3,4-dihydro-6-methyl-2,4-dioxo-2H-pyran-3-ylidene)ethanolate
Supplier	LONZA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to yellow, odourless powder
Content %	~100 (H ₂ O content 8.65)
Melting point °C	110–112
Bulk density kg/m ³	500–550
Ignition temperature °C	360
pH value (100 g/l H ₂ O)	8.8–9.3 at 20°C
Stability	stable under normal conditions
Solubility g/l (25°C)	330 in H ₂ O, 10 in ethanol, 497.4 in propylene glycol, 110.80 in methanol; insoluble in acetone, benzene
Toxicity data (8.1.8. and 8.1.8a.)	
LD ₅₀ oral	~1000 mg DHA/kg rat ~500 mg DHA-Na/kg rat

Both active agents do not cause skin irritation, however they are mildly irritant to eyes.
Sensitization has not been observed.
Ames test and chromosome test were negative.

Antimicrobial effectiveness/applications (8.1.8/8.1.8a.)

DHA is more effective against mould producing fungi and yeasts than against bacteria. The efficacy is restricted to media with acid pH value; contrary to other acids used as preservatives DHA is, however, still effective at a pH as high as 6. In comparison to sodium benzoate (8.1.9a) is DHA-Na more effective against *Aspergillus niger*, *Penicillium glaucum* and *Saccharomyces cerevisia*. Optimum efficacy is produced at pH 4–6. Using DHA/DHA-Na as a preservative for the in-can protection of aqueous functional fluids it is recommended to combine it with a bacteriostatic agent, to broaden the spectrum of protection.

In the EC list of preservatives allowed for the protection of cosmetics DHA is listed with a maximum concentration of 0.6% (prohibited in sprays). – Percentage of use in US cosmetic formulations: 0.80% DHA; 1.11% DHA sodium salt.

Table 79 Minimum inhibition concentrations (MIC) of dehydroacetic acid (Source: LONZA)

Test organism (10 ⁶ cfu/ml)	MIC (mg/l, 72 h)
<i>Staphylococcus aureus</i>	10.000
<i>Bacillus subtilis</i>	5.000
<i>Escherichia coli</i>	10.000
<i>Pseudomonas aeruginosa</i>	> 20.000
<i>Candida albicans</i>	100
<i>Aspergillus oryzae</i>	1.250
<i>Aspergillus niger</i>	200
<i>Penicillium glaucum</i>	200

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.9. Benzoic acid
Chemical formula	C ₇ H ₆ O ₂
Structural formula	C ₆ H ₅ -COOH
Molecular mass	122.12
CAS-No.	65-85-0
EC-No.	200-618-2; EEC-no. 1; permitted food additive (E 210)
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	Classified as GRAS in the USA
Supplier	benzene carboxylic acid, phenyl formic acid BAYER, BF GOODRICH-KALAMA, DSM, LIQUID CARBONIC, MITSUBISHI CHEMICAL, VELSICOL CHEMICAL
Chemical and physical properties	
Appearance	white, monoclinic flakes or needles, or crystalline powder
Content (%)	> 99
Boiling point/range °C (101 kPa)	250
Melting point °C	122 (sublimation at 100°C)
Density g/ml (20°C)	1.32
Vapour pressure hPa (132°C)	13.3
Flash point °C	121
Auto ignition temperature	532
Dissociation constant	6.46 × 10 ⁻⁵
pKa value	4.2
pH value (3 g/l H ₂ O)	3.0 at 20°C
Log POW	1.87
Solubility g/l (20°C)	2.9 in H ₂ O, 435 in ethanol, 10–20 in fatty oils
Chemical name	8.1.9a. Benzoic acid sodium salt
Chemical formula	C ₇ H ₅ NaO ₂
Structural formula	C ₆ H ₅ -COO ⁻ Na ⁺
Molecular mass	144.11
CAS-No.	532-32-1
EC-No.	208-534-8; E 211
Synonym/common name	sodium benzoate
Chemical and physical properties	
Appearance	white, crystalline powder
Content %	> 99
Melting point	> 400
Solubility g/l	500 in H ₂ O, 13 in ethanol, insoluble in ether and other non polar solvents and lipids
pH value (100 g/l H ₂ O)	9 at 20°C

Chemical name	8.1.9b. Benzoic acid potassium salt
Chemical formula	$C_7H_5KO_2$
Structural formula	$C_6H_5-COO^-K^+$
Molecular mass	160.2
CAS-No.	582-25-2
EC-No.	209-481-3; E 212
Synonym/common name	potassium benzoate
Chemical and physical properties	
Appearance	white, crystalline powder
Content	99
Melting point °C	> 300
Solubility	highly soluble in water
pH value (100 g/l H ₂ O)	8.5 at 20°C

Chemical name	8.1.9c. Benzoic acid calcium salt
Chemical formula	$C_{14}H_{10}O_4Ca$
Structural formula	$(C_6H_5-COO^-)_2Ca^{2+}$
Molecular mass	282.31
CAS-No.	2090-05-3
EC-No.	208-534-8; E 213
Synonym/common name	calcium benzoate
Appearance	white, crystalline powder

Toxicity data of benzoic acid (8.1.9.)

LD ₀ oral	500 mg/kg man
LD ₅₀ oral	2530 mg/kg rat
intraperitoneal	1600 mg/kg rat
intravenous	1700 mg/kg rat
dermal	> 10 g/kg rabbit
LC ₅₀ on inhalation	> 26 mg/m ³ (1 h) for rats
Non-irritant to skin, however, irritant to eyes and the upper respiratory tracts.	
ADI value: 0–5 mg/kg body weight.	
Ecotoxicity (source: BAYER AG):	
EC ₀ for <i>Pseudomonas putida</i>	480 mg/l
LC ₅₀ for <i>Leuciscus idus</i>	460 mg/l
Degradability according to the closed bottle test: Good.	

Antimicrobial effectiveness/applications

Benzoic acid is a naturally occurring acid (detectable in several berries and fruit) which exhibits antimicrobial activity in its undissociated form only. According to its pK_a value it is therefore significantly effective only in acid formulations at pH 4.5 or below (optimum pH 4–2.5). At pH 6 only 1.52% non-ionized benzoic acid is available. Yeasts are especially sensitive to benzoic acid; the antibacterial activity of benzoic acid is superior to that of sorbic acid (8.1.5.).

Sodium benzoate is an especially inexpensive preservative agent and probably the most extensive used food preservative (E 211) in the world. Benzoic acid, its salts and esters are permitted for the preservation of cosmetic products; maximum authorized concentration: 0.5% (acid) according to the EC positive list. – Percentage of use in US cosmetic formulations: 0.66% benzoic acid; 0.34% sodium benzoate. Sometimes benzoic acid or sodium benzoate are also applied as preservatives in acid technical functional fluids, e.g. polymer emulsions, especially in those coming in contact with foodstuffs. In acid two-phase systems (emulsions) it has to be taken into consideration that benzoic acid shows a marked tendency to migrate into the organic (oil) phase, so that its activity in the water phase, where the microbes are vegetating, is decreased. Losses of activity are also observed in the presence of non-ionic detergents, quaternary ammonium compounds, proteins and glycerine. Propylen glycol intensifies the efficacy of benzoic acid.

Table 80 Minimum inhibition concentrations (MIC) of benzoic acid in nutrient agar at pH 6 (Wallhäusser, 1984)

Test organism	MIC (mg/litre)
<i>Staphylococcus aureus</i>	20
<i>Escherichia coli</i>	160
<i>Klebsiella pneumoniae</i>	160
<i>Pseudomonas aeruginosa</i>	160
<i>Pseudomonas fluorescens</i>	160
<i>Pseudomonas cepacia</i>	160
<i>Candida albicans</i>	1200
<i>Aspergillus niger</i>	1000
<i>Penicillium notatum</i>	1000

Microbicide group (substance class)

8.1. ORGANIC ACIDS

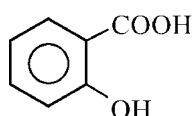
Chemical name

8.1.10. Salicylic acid

Chemical formula

 $C_7H_6O_3$

Structural formula



Molecular mass

138.13

CAS-No.

69-72-7

EC-No.

200-712-3; EEC-no. 3

EPA TSCATS

Data base, Jan. 2001

Synonym/common name

2-hydroxybenzoic acid

Supplier

J. T BAKER INC., KALAMA CHEM. CORP., MERCK Inc., RdH, SIGMA-ALDRICH

Chemical and physical properties

Appearance

white, crystalline powder

Content (%)

~99

Boiling point/range °C (2.66 kPa)

211

Melting point °C

157–159

Density g/ml (20°C)

1.44

Vapour pressure hPa (114°C)

1.33

Flash point °C

157

Dissociation constant

 1.07×10^{-3}

pKa value

2.97

pH (saturated solution in H₂O)

2.4

Stability

sublimation at 76°C; light sensitive; thermal degradation to phenol and CO₂; discolouration with iron salts

Solubility g/l (20°C)

2.2 in H₂O, 370 in ethanol, 17 in glycerine, 12 in oils

Toxicity data

LD₅₀ oral

891 mg/kg rat

dermal

> 2000 mg/kg rat

interperitoneal

157 mg/kg rat

intravenous

184 mg/kg mouse

LC₅₀ on inhalation> 900 mg/m³ (1 h) for rats

Skin irritation (test with rabbits): 500 mg (24 h): mild.

Eye irritation (test with rabbits): 100 mg severe.

Antimicrobial effectiveness/applications

The efficacy of salicylic acid is in first line directed against yeasts and moulds, but its activity against bacteria is also worth a mention as it is more pronounced than that of benzoic acid. However, because of its low pK_a value salicylic acid can only be used for the preservation of highly acid products. As a food preservative it has been abandoned because of its toxicity. Still of some importance is the application of salicylic acid for the protection of cosmetics and pharmaceutical products. In the EC list of preservatives allowed for cosmetics, salicylic acid and

its salts are mentioned with a maximum authorized concentration of 0.5%. Percentage of use in US cosmetic formulations: 0.11%.

8.1.11. Alkyl 4-hydroxybenzoates.

Synonym/common name

Phenol-4-carboxylic acid esters, p-hydroxybenzoates, p-hydroxybenzoic acid alkyl esters, Parabens

p-Hydroxybenzoic acid alkyl esters, universally known as "Parabens", are listed under "Acids" and not under "Esters" because of their exceptional acid character. The starting product for the synthesis of p-hydroxybenzoic acid alkyl esters, p-hydroxybenzoic acid (PHBA) is a naturally occurring acid, e.g. in raspberries and blackberries. PHBA alkyl esters are relatively stable chemicals; hydrolysis takes place only under drastic conditions (e.g. pH 10 or pH 1). At pH 4 the PHBA methyl and ethyl ester are stable between 40 and 121°C. Lipid solubility and efficacy of PHBA alkyl esters increase with increasing length of the alkyl chain, reaching a maximum at the butyl ester. The PHBA methyl ester distinguishes itself with optimal water solubility (0.25%), which decreases with increasing alkyl chain length. The lack of good water solubility is a certain limitation for the application of PHBA alkyl esters as preservatives. An advantage over benzoic acid and other preservative acids is the relatively high pKa value (approx. 8.5) of the PHBA alkyl esters, which means that they exhibit antimicrobial activity even at neutral and slightly higher pH values (see Figure 16).

PHBA alkyl esters are more effective against moulds and yeasts than against bacteria; nevertheless they are useful for the control of bacterial growth (see Table 81). As membrane active microbicides, their primary mode of action is based on the inhibition of nutrient transport into the microbial cell. Because of the following favourable properties PHBA alkyl esters most closely approach the ideal preservative:

- broad spectrum of effectiveness covering moulds and bacteria,
- effective at low concentrations (0.05–0.2%),
- stable, odourless, colourless,
- not significantly influencing the taste of food,
- active over a wide range of pH and temperature,
- low toxicity, non-irritant parenterally.

Applications of PHBA alkyl esters

These are mainly in the preservation of foods, pharmaceuticals and cosmetics, although there is a certain sensitization potential. PHBA alkyl esters are also used for the in-can protection of technical functional fluids, when one looks for particular odourless and colourless preservatives. Using PHBA alkyl esters for the protection of non-ionic surfactant solutions one has to bear in mind that binding effects may occur which reduce the antimicrobial efficacy. This is valid for anionic alkaline detergent solutions, too. Macromolecules, e.g. saccharosesters, polyoxy-40-stearate, polyvinyl-pyrrolidone, methylcellulose and carboxymethylcellulose, are also able to

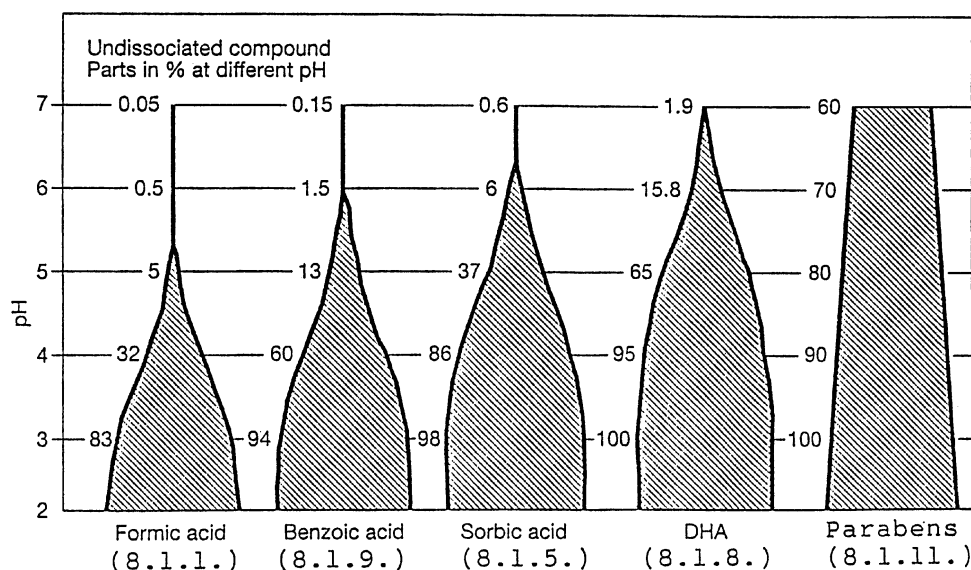


Figure 16 Undissociated portions of different preservative acids at different pH values.

Table 81 Minimum inhibition concentrations (MIC in %) of p-hydroxybenzoic acid alkyl esters (Source: CLARIANT-NIPA)

Test organism	Methyl ester	Ethyl ester	Propyl ester	Butyl ester
Gram-negative Bacteria				
<i>Pseudomonas aeruginosa</i>	0.2	0.10	0.08	> 0.02
<i>Escherichia coli</i>	0.1	0.05	0.04	> 0.02
<i>Klebsiella aerogenes</i>	0.075	0.05	0.04	0.02
<i>Klebsiella pneumoniae</i>	0.1	0.05	0.025	0.015
<i>Serratia marcescens</i>	0.075	0.05	0.04	0.2
<i>Proteus vulgaris</i>	0.10	0.06	0.025	0.015
<i>Salmonella enteritidis</i>	0.15	0.05	0.04	> 0.02
<i>Salmonella typhi</i>	0.15	0.10	0.06	> 0.02
Gram-positive Bacteria				
<i>Staphylococcus aureus</i>	0.15	0.07	0.04	0.015
<i>Streptococcus haemolyticus</i>	0.1	0.06	0.04	0.015
<i>Bacillus cereus</i>	0.075	0.025	0.025	0.015
<i>Bacillus subtilis</i>	0.10	0.10	0.025	0.015
<i>Lactobacillus buchneri</i>	0.10	0.06	0.025	0.01
Yeasts and Moulds				
<i>Candida albicans</i>	0.1	0.07	0.013	0.013
<i>Saccharomyces cerevisiae</i>	0.1	0.05	0.013	0.005
<i>Aspergillus niger</i>	0.1	0.04	0.02	0.020
<i>Penicillium digitatum</i>	0.05	0.025	0.006	> 0.005
<i>Rhizopus nigricans</i>	0.05	0.025	0.013	0.005

reduce the effectiveness of PHBA alkyl esters. In nonionic formulations the activity of PHBA alkyl esters is increased by the addition of 2–5% propylene glycol. PHBA methyl and propyl esters are more widely used than the others and frequently in combination to broaden the spectrum of activity. The solubility of PHBA alkyl esters in preparations to be protected can be promoted by heating. In cases where preparations do not tolerate heating one can incorporate the sodium salts of PHBA alkyl esters which are highly soluble in water or aqueous formulations. An increase in pH caused through the alkaline reaction of the sodium salts can be corrected subsequently by the addition of an acidulant, e.g. citric acid or acetic acid.

PHBA esters (methyl, ethyl, propyl and butyl ester) and their salts are listed in the EC list of preservatives permitted for the in-can protection of cosmetics with a maximum authorized concentration of 0.4% (acid) for one ester and 0.8% (acids) for mixtures of esters (EEC-no. 12). Maximum permitted concentrations in Japan: 1.0. %. Status for US cosmetic formulations: Safe as used.

Microbicide group (substance class)

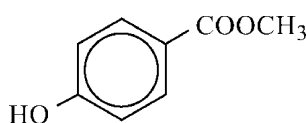
Chemical name

Chemical formula

Structural formula

8.1.11. ALKYL 4-HYDROXYBENZOATES

8.1.11.1. Methyl 4-hydroxybenzoate

 $C_8H_8O_3$ 

Molecular mass

CAS-No.

EC-No.

152.2

99-76-3

202-785-7; EEC-no.12

food additive E218

EPA TSCA

Synonym/common name

Section 8(B) Chemical Inventory

p-hydroxybenzoic acid methylester, methyl

p-hydroxybenzoate,

p-carbmethoxyphenol, Methylparaben

Supplier

BAYER, CLARIANT-NIPA, KALAMA CHEMICALS

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

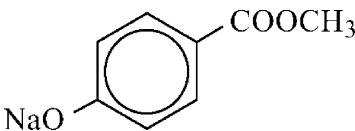
Melting point °C

white, crystalline, nearly odourless hygroscopic powder

min. 99.5

298.6

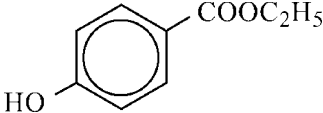
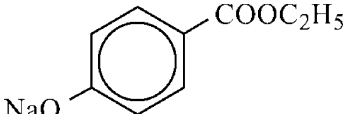
min. 125

Bulk density g/l	700
Vapour pressure hPa (20°C)	4.51×10^{-6} (at 50°C: 3.48×10^{-4})
Log POW	1.96
pH value (~1% in H ₂ O)	neutral to slightly acidic
Stability	in water up to pH 8; hydrolysis in alkaline solutions
Solubility g/l (25°C)	~2.5 in H ₂ O (at 80°C: ~20), 600 in ethanol, 320 in diethylene glycol, 280 in propylene glycol, 10 in glycerine, 20 in linseed oil, 30 in olive oil, 5 in paraffin oil
Toxicity data	
LD ₅₀ oral	> 8000 mg/kg mouse 6000 mg/kg rabbit 3000 mg/kg guinea pig
LD ₅₀ intraperitoneal	960 mg/kg mouse
LD ₅₀ subcutaneous	> 500 mg/kg rat 1200 mg/kg mouse
Results of skin and eye irritation tests with rabbits: slightly irritant.	
Exotoxicity (source: BAYER):	
The microbicide is ready biodegradable (above 90% in the closed bottle test).	
EC ₀ for <i>Pseudomonas fluorescens</i> :	500 mg/l
LC ₀ for fish (<i>Leuciscus idus</i>):	50 mg/l (48 h)
Chemical name	8.1.11.1a. Sodium methyl 4-hydroxybenzoate
Chemical formula	C ₈ H ₇ O ₃ Na
Structural formula	
Molecular mass	174.1
CAS-No.	5026-62-0
EC-No.	225-714-1; EEC-no.12
Synonym/common name	food additive 219
Supplier	sodium 4-(methoxycarbonyl)phenolate BAYER, CLARIANT-NIPA
Chemical and physical properties	
Appearance	white, almost odourless powder
Content (%)	> 99.5 (PHBA-ester content: 87)
Bulk density g/l	~700
pH value (1 g/l H ₂ O)	approx. 10 at 20°C
Stability	aqueous solutions are not storable because of the alkalinity of such solutions
Solubility g/l (25°C)	330 in H ₂ O, 20 in ethanol, ~250 in propylene glycol, ~500 in glycerine, virtually insoluble in oils
Toxicity data (source: CLARIANT-Nipa)	
LD ₅₀ oral	> 5000 mg/kg rat ~2000 mg/kg mouse
Slightly irritant on the skin; risk of serious damage to eyes (as a result of the alkalinity of the product). Inhalation of dust causes slight irritation of the respiratory tract.	
Ecotoxicity:	
LC ₀ for <i>Leuciscus idus</i>	100 mg/l (48 h)

Antimicrobial effectiveness/applications

The MIC in Table 81 demonstrate the spectrum of effectiveness of methyl 4-hydroxybenzoate and classify the agent as a preservative of medium strength which in comparison to other alkyl 4-hydroxybenzoates is superior

by its water solubility. Methyl 4-hydroxybenzoate and its sodium salt are used to preserve cosmetic and pharmaceutical preparations, but technical products too. A combination of the preservative with propyl 4-hydroxybenzoate (8.1.11.3.) is often the best solution. The use of methyl 4-hydroxybenzoate as a preservative for foodstuffs, however, has not been approved in all countries.

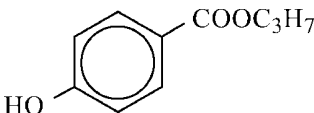
Microbicide group (substance class)	8.1.11. ALKYL 4-HYDROXYBENZOATES
Chemical name	8.1.11.2. Ethyl 4-hydroxybenzoate
Chemical formula	$C_9H_{10}O_3$
Structural formula	
Molecular mass	166.2
CAS-No.	120-47-8
EC-No.	204-399-4; food additive E 214; EEC-no.12
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	p-hydroxybenzoic acid ethyl ester, p-carbethoxyphenol, Ethylparaben
Supplier	BAYER, CLARIANT-NIPA, KALAMA CHEMICALS
Chemical and physical properties	
Appearance	white, crystalline, nearly odourless powder
Content (%)	min. 99.5
Boiling point/range °C (101 kPa)	297–298
Melting point °C	115–119
Bulk density g/l	600
Log POW	2.47
pH value (1 g/l in H ₂ O)	approx. 7
Stability	stable under normal conditions; not storable in aqueous solution at pH > 8
Solubility g/l (25°C)	1.2 in H ₂ O (at 80°C: approx. 8.6) 600 in ethanol, 250 in diethylene glycol, 250 in propylene glycol, 6 in glycerine, 22 in linseed oil, 30 in olive oil, 5 in paraffin oil
Toxicity data	
LD ₅₀ oral	3000 mg/kg mouse 5000 mg/kg rabbit 2000 mg/kg guinea pig
LD ₅₀ intraperitoneal	520 mg/kg mouse
Results of skin and eye irritation tests with rabbits:	slightly irritant.
Ecotoxicity (source: BAYER)	
The microbicide is ready biodegradable (above 90% in the closed bottle test).	
EC ₀ for <i>Pseudomonas aeruginosa</i>	1000 mg/l
LC ₀ for fish (<i>Leuciscus idus</i>)	20 mg/l (96 h)
Chemical name	8.1.11.2a. Sodium ethyl 4-hydroxybenzoate
Chemical formula	$C_9H_9O_3Na$
Structural formula	
Molecular mass	188.2
CAS-No.	35285-68-8
EC-No.	252-487-6, food additive E 215; EEC-no.12
Synonym/common name	sodium 4-(ethoxycarbonyl)phenolate
Supplier	BAYER, CLARIANT-NIPA

Chemical and physical properties

Appearance	white, almost odourless, hygroscopic powder
Content (%)	min. 99.5 (PHBA-ester content: 87.4)
Bulk density g/l	600
pH value (1 g/l in H ₂ O)	approx. 10 at 20°C
Stability	no decomposition up to 100°C; aqueous solutions are not storable because of the alkalinity of such solutions
Solubility g/l (25°C)	500 in H ₂ O, 20 in ethanol, ~300 in propylene glycol, ~500 in glycerine, virtually insoluble in oils
Toxicity data (source: CLARIANT-NIPA)	
LD ₅₀ oral	> 2000 mg/kg mouse
Slightly irritant on the skin; risk of serious damage to eyes (as a result of the alkalinity of the product). Inhalation of dust causes slight irritation of the respiratory tract.	

Antimicrobial effectiveness/applications

Ethyl 4-hydroxybenzoate is a highly effective preservative with moderately good solubility in water. The spectrum of effectiveness is demonstrated by the MIC in Table 81. As a food preservative ethyl 4-hydroxybenzoate is often used in combination with propyl 4-hydroxybenzoate (8.1.11.3.); thus it is possible in most cases to use smaller overall amounts of alkyl 4-hydroxybenzoate than would otherwise be necessary. The favourable properties of ethyl 4-hydroxy benzoate makes it also highly suitable for preserving cosmetics and pharmaceuticals; most frequently mixtures of alkyl 4-hydroxybenzoates are used.

Microbicide group (substance class)	8.1.11. ALKYL 4-HYDROXYBENZOATES
Chemical name	8.1.11.3. n-Propyl 4-hydroxybenzoate
Chemical formula	C ₁₀ H ₁₂ O ₃
Structural formula	
Molecular mass	180.2
CAS-No.	94-13-3
EC-No.	202-307-7; EEC-no.12
EPA TSCA	food additive E 216
Synonym/common name	Section 8(B) Chemical Inventory p-hydroxybenzoic acid propyl ester, p-carbpropoxyphenol, Propylparaben
Supplier	BAYER, CLARIANT-NIPA, KALAMA CHEMICALS
Chemical and physical properties	
Appearance	white, crystalline, nearly odourless powder
Content (%)	min. 99.5
Melting point	96
Bulk density g/l	650
Log POW	3.04
pH value (0.4 g/l H ₂ O)	approx. 7 at 20°C
Stability	stable under normal conditions; not storable in aqueous solution at pH > 8
Solubility g/l (25°C)	~0.4 in H ₂ O (at 80°C ~1.3), 750 in ethanol, 350 in diethylene glycol, 250 in propylene glycol, 68 in linseed oil, 52 in olive oil, 10 in paraffin oil

Toxicity data

LD ₅₀ oral	6332 mg/kg mouse
subcutaneous	1650 mg/kg mouse
intraperitoneal	200 mg/kg mouse

Slightly irritant on skin and eyes.

Ecotoxicity (source: BAYER):

The microbicide is ready biodegradable (above 95% in the closed bottle test).

EC₀ Escherichia coli 500 mg/l

EC₀ for Pseudomonas fluorescens 1000 mg/l

LC₀ for fish (Leuciscus idus) 5 mg/l (48 h)

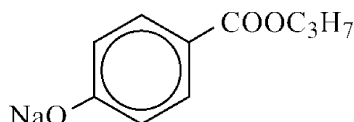
Chemical name

8.1.11.3a. Sodium n-propyl 4-hydroxybenzoate

Chemical formula

C₁₀H₁₁O₃Na

Structural formula



Molecular mass

202.2

CAS-No.

35285-9-9

EC-No.

252-488-1; EEC-no.12

food additive E 217

Synonym/common name

sodium 3-(propoxycarbonyl)phenolate

Supplier

BAYER, CLARIANT-NIPA

Chemical and physical properties

Appearance

white, almost odourless powder

Content (%)

min. 99 (PHBA-ester content: 88.2)

Bulk density g/l

~600

Log POW

3.04

pH value (1 g/l H₂O)

approx. 10 at 20°C

Stability

stable under normal conditions; aqueous solutions are not storable; the alkalinity of such solutions causes hydrolysis to inactive p-hydroxybenzoic acid

Solubility g/l (25°C)

500 in H₂O, 20 in ethanol, ~400 in propylene glycol, ~500 in glycerine, virtually insoluble in oils

Toxicity data (source: CLARIANT-NIPA)

LD₅₀ oral > 2000 mg/kg mouse

Slightly irritant on the skin; risk of serious damage to eyes. Inhalation causes slight irritation of the respiratory tract.

Antimicrobial effectiveness/application

n-Propyl 4-hydroxybenzoate is particularly effective in inhibiting the proliferation of fermentative yeasts (see Table 81). Applying the propylester one should regard the fact that it tends to migrate into organic (oil) phases (see log POW). In general n-propyl 4-hydroxybenzoate is used in conjunction with methyl 4-hydroxybenzoate (8.1.11.1.) for the in-can protection of cosmetic and pharmaceutical products and in combination with ethyl 4-hydroxybenzoate (8.1.11.2.) to protect foodstuffs.

Microbicide group (substance class)

8.1.11. ALKYL 4-HYDROXYBENZOATES

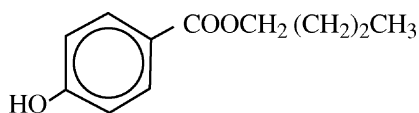
Chemical name

8.1.11.4. n-Butyl 4-hydroxybenzoate

Chemical formula

C₁₁H₁₄O₃

Structural formula



Molecular mass	194.23
CAS-No.	94-26-8
EC-No.	202-318-7
EPA-TSCA	Section 8(B) Chemical Inventory
Synonym/common name	p-hydroxybenzoic acid butylester, phenol-4-butylcarboxylate
Supplier	CLARIANT-NIPA, MIDORI KAGAKU CO., SIGMA-ALDRICH

Chemical and physical properties

Appearance	white, crystalline, odourless or almost odourless powder
Content %	~100
Melting point °C	68–71
Log POW	3.57
Stability	stable and effective between pH 3 and 9.5; hydrolysis at lower or higher pH values, increasing with temperature
Solubility g/l (20°C)	0.2 in H ₂ O; highly soluble in ethanol and other organic solvents

Toxicity data

LD ₅₀ oral	13200 mg/kg mouse
intrapertoneal	230 mg/kg mouse
Can cause slight irritation at the skin, eyes and respiratory tract.	

Chemical name	8.1.11.4a. Sodium n-butyl-4-hydroxybenzoate
Chemical formula	C ₁₁ H ₁₃ NaO ₃
Molecular mass	216.22
CAS-No.	36457-20-2
EC-No.	253-049-7

Chemical and physical properties

Appearance	white, almost odourless powder
Content %	~100
Solubility g/l (20°C)	500 in H ₂ O
pH value (1 g/l at 20°C)	approx. 10
Stability	the alkalinity of aqueous solutions of the sodium salt stimulates the hydrolytic ester cleavage, especially at elevated temperatures

Toxicity data

LD50 oral	950 mg/kg mouse
The alkaline substance is slightly irritant on the skin; it may seriously damage the eyes.	

Ecotoxicity (source: CLARIANT-NIPA):

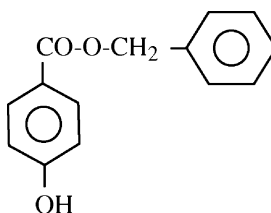
LC ₀ for <i>Leuciscus idus</i>	100 mg/l (48 h)

Antimicrobial effectiveness/applications

In comparison with the lower p-hydroxybenzoic acid alkyl esters the butyl ester is far and away the most effective one and presents an equalized spectrum of effectiveness (see Table 81). However, the application of the butyl ester as a preservative in cosmetics, toiletries and pharmaceuticals is, in spite of its high activity, limited because of its low water solubility and high log POW. But combinations of p-hydroxy-benzoic acid butyl ester with the lower alkyl esters perform excellently.

Microbicide group (substance class)	8.1.11. ALKYL 4-HYDROXYBENZOATES
Chemical name	8.1.11.5. Benzyl 4-hydroxybenzoate
Chemical formula	C ₁₄ H ₁₂ O ₃

Structural formula



Molecular mass	228.25
CAS-No.	94-18-18
EC-No.	202-311-9
Synonym/common name	p-hydroxybenzoic acid benzyl ester, phenol-4-benzylcarboxylate
Supplier	FLUKA, MERCK

Chemical and physical properties

Appearance	white crystalline powder
Content (%)	~100
Melting point °C	108–113
Stability	stable between pH 3 and 9 under normal conditions
Solubility g/l (25°C)	0.06 in H ₂ O, 700 in ethanol, 5 in mineral oil

Toxicity data:

Although the CIR Expert Panel of CTFA in its final report states that PHBA benzyl ester in concentrations which are normally applied in cosmetics, can be regarded as safe (*J. amer. Coll. Toxicol.*, 5 (1986) 301), the benzyl ester is not listed in the EC list of preservatives allowed for the in-can protection of cosmetics.

Antimicrobial effectiveness/applications

Although highly effective the use of PHBA benzyl ester as a preservative is strongly limited because of its extremely poor water-solubility. Application is therefore only in combination with other PHBA esters.

Table 82 Minimum inhibition concentration (MIC) of PHBA benzyl ester in nutrient agar according to Wallhäusser (1984)

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	160
<i>Klebsiella pneumoniae</i>	160
<i>Pseudomonas aeruginosa</i>	160
<i>Pseudomonas cepacia</i>	160
<i>Pseudomonas fluorescens</i>	160
<i>Staphylococcus aureus</i>	120
<i>Candida albicans</i>	250
<i>Aspergillus niger</i>	1000
<i>Penicillium notatum</i>	500

Microbicide group (substance class)

Chemical name

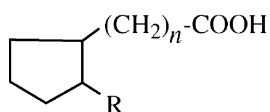
Chemical formula

Structural formula

8.1. ORGANIC ACIDS

8.1.12. Naphthenic acid, tech.

unspecified



R = H, CH₃, cyclopentyl
n = 0 – 60

CAS-No.	1338-24-5
EC-No.	215-662-8
Supplier	DURHAM CHEMICALS, SIGMA-ALDRICH

Chemical and physical properties

Appearance	clear, deep brownish liquid
Density g/ml (20°C)	0.95
Refractive index nD (20°C)	1.48
Flash point °C	> 100
Chemical name	8.1.12a. Copper naphthenate, unspecified
CAS-No.	1338-02-9
EC-No.	215-657-0
Synonym	naphthenic acids copper salts
Appearance	grey-green paste
Copper content %	14–15
Solubility	virtually insoluble in water; soluble in organic solvents

Antimicrobial effectiveness/applications

Naphthenic acids are a by-product of oil refining. They are monocarboxylic acids of cyclic alkanes according to the structure indicated above.

The salts of the alicyclic naphthenic acids are sparingly soluble in water and regarded as metallic soaps. Copper naphthenate is especially active against a broad range of fungi and therefore mainly used in wood preservatives. However, it has to be kept in mind that there are copper-tolerant fungi which are able, to biodegrade wood treated with copper-based preservatives (De Groot et al., 1999). Bratt et al. (1992) assessed copper naphthenate in comparison to zinc naphthenate, copper-8-hydroxyquinolate (13.3a), tributyl tin oxide (19.5) and pentachlorophenol (7.5.4.) for their ability to preserve soft wood in a tropical environment (Queensland, Australia). Only wood blocks treated with copper naphthenate showed consistently lower levels of biodeterioration when compared with untreated control specimens.

Copper naphthenate has been employed also for the fungicidal, non-leachable treatment of textiles (e.g. tents, sandbags, etc.), paper and cardboard. For this applications solutions of copper naphthenate in organic solvents are preferably used.

Microbicide group (substance class)	8.1. ORGANIC ACIDS
Chemical name	8.1.13. n-Dodecanoic acid
Chemical formula	$C_{12}H_{24}O_2$
Structural formula	$H_3C-(CH_2)_{10}-COOH$
Molecular mass	200.32
CAS-NO.	143-07-7
EC-No.	205-582-1
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	Lauric acid, 1-undecanecarboxylic acid
Supplier	SIGMA-ALDRICH

Chemical and physical properties

Appearance	colourless needles
Content (%)	> 99
Boiling point/range °C (0.13 kPa)	131
Melting point °C	44–45
Density g/ml (20°C)	0.868
Vapour pressure hPa (20°C)	66.5
Flash point °C	165
Stability	stable under normal conditions
Solubility	virtually soluble in H ₂ O, soluble in alcohols, ether

Toxicity data

LD ₅₀ oral	12 g/kg rat
LD ₅₀ intravenous	131 mg/kg mouse
Mildly irritant to skin and eyes (test with rabbits).	

Antimicrobial effectiveness/application

Glycerine esters of Lauric acid are an essential part of Lauric oil which naturally occurs, e.g. in palm-oil and coco-nutoil: the latter contain in contrary to other plant-oils high amounts of glyceryl laureates (40–50%) and are used for the production of Lauric acid by saponification, followed by distillation. The antimicrobial activity of Lauric acid is negligible. The acid is mentioned here, as it is part of the microbicide Lauricidin (9.8. = glyceryl monolaurate).

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Density g/ml (20°C)

Vapour pressure hPa (20°C)

Dissociation constant

pKa value

Stability

Solubility g/l (20°C)

Toxicity data

LD₀ oral

dermal

LD₅₀ oralLD₅₀ subcutaneous

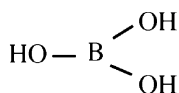
intravenous

Mildly irritant to skin and eyes.

Rapid absorption through the skin, slow excretion, danger of accumulation. Mutagenicity was not demonstrable.

8.2. INORGANIC ACIDS

8.2.1. Boric acid

H₃BO₃

61.84

10043-35-3

233-139-2-Notif.-No.: N482

Data base, Jan. 2001

ortho-boric acid, trihydroxyborone

RdH

white, crystalline, odourless powder

~99

171

1.52

3.46

 7×10^{-10}

9.14

volatile with water steam

approx. 55 in H₂O, approx. 50 in ethenol

429 mg/kg man

200 mg/kg woman

2430 mg/kg man

2660 mg/kg rat

3450 mg/kg mouse

1400 mg/kg rat

1330 mg/kg rat

Chemical name

Chemical formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content %

Melting point °C

8.2.1a. Borax

B₄Na₂O₇·10 H₂O

381.37

1303-96-4

215-540-4

disodium tetraborate, sodium borate

RdH

colourless, odourless, white crystals or powder

> 99 (boric acid content 52.3–54.3)

878 after dehydration

Density g/ml (20°C)	1.73
pH (0.4% in H ₂ O at 25°C)	9.1–9.2
Stability	dehydration at 400°C
Solubility	soluble in hot water and glycerine; insoluble in ethanol

Antimicrobial effectiveness/applications

Boric acid's spectrum of activity is rather selective and covers mainly yeasts with lower efficacy against moulds and bacteria. Although boric acid is only effective in its undissociated state, it is still active even at neutral pH because of its relatively high pK_a value (9.14). Boric acid acts as an enzyme inhibitor, especially blocking enzymes in the metabolism of phosphate.

In the EC list of preservatives, provisionally allowed for cosmetics, boric acid is listed with a maximum authorized concentration of 0.5% for products for oral hygiene and 3% for other products. As a food preservative boric acid is no longer of importance because of its toxicological properties.

For sapstain treatment (protection of freshly cut and sawn timber against staining fungi and superficial moulds) borax is used in mixture with other fungicides, formerly with sodium pentachlorophenate (7.5.4.), today with sodium phenylphenolate (7.4.1.) or with quaternary ammonium compounds (18.1).

Chemical name	8.2.1b. Barium metaborate monohydrate Metaborates derive from metaboric acid (O = B-OH) which is boric acid after the loss of 1 mol H ₂ O.
Chemical formula	B ₂ H ₂ BaO ₅
Structural formula	O = B-O-Ba-O-B = O·H ₂ O
Molecular mass	240.97
CAS-No.	13701-59-2
EC-No.	237-222-4
Synonym/common name	barium diboron tetraoxide
Supplier	BUCKMAN
Chemical and physical properties	
Appearance	white odourless powder
Content (%)	90–100 (Ba content: ~57; boron content: ~9)
Fusion temperature °C	900–1050
Density g/ml (25°C)	3.25–3.35
pH (saturated solution)	9.5–11
Stability	in the presence of sulphates conversion to insoluble and innocuous barium sulphate (BaSO ₄)
Solubility g/l	3–4 in H ₂ O; insoluble in organic solvents
Toxicity data (source: BUCKMAN)	
LD ₅₀ oral	850 mg/kg rat
LD ₅₀ dermal	> 2000 mg/kg rabbit
LC ₅₀ on inhalation	2.54 mg/l (4 h) for rats
Irritant to skin and eyes. Non-sensitizer for skin.	
Ecotoxicity:	
LC ₅₀ for Rainbow trout	> 62 mg/l (96 h)
LC ₅₀ for Daphnia magna	20.3 mg/l (48 h)

Antimicrobial effectiveness/applications

Barium metaborate can be regarded as a multifunctional pigment when used in paints. It keeps an alkaline environment in the paint can and the paint film, thus inhibiting mould growth and corrosion. The addition rates for mould resistant coatings are, however, relatively high (5–20%). Not all types of polymer emulsions tolerate such high salt concentrations. The water-solubility of barium metaborate also is not negligible and has to be taken into consideration if the compound is used in exterior coatings as a paint film fungicide.

Microbicide group (substance class)	8.2. INORGANIC ACIDS
Chemical name	8.2.2. Sulphurous acid anhydride = Sulphur dioxide
Chemical formula	SO ₂
Structural formula	$\text{O}=\text{S}=\text{O} \xrightarrow{+\text{H}_2\text{O}} \text{HO}-\underset{\text{O}}{\underset{\parallel}{\text{S}}}-\text{OH}$ Sulphurous acid
Molecular mass	64.06
CAS-No.	7446-09-5
EC-No.	231-195-2; EEC-no. 9; food additive E 220
EPA TSCATS	Data base, Jan. 2001 classified "GRAS" in the USA
Synonym/common name	sulphurous anhydride
Supplier	BASF, RdH
Chemical and physical properties	
Appearance	colourless, non-flammable gas with a pungent odour, which under pressure forms a liquid
Content (%)	~100
Boiling point/range °C (101 kPa)	-10.08
Melting point °C	-75.5
Vapour density g/l	2.26
Vapour pressure hPa (20°C)	2.37
pKa values	1.8/6.9
Stability	highly reactive and reductive; hygroscopic
Sulphites	sodium sulphite (Na ₂ SO ₃), M = 126.04 (CAS-no. 77-57-83-7; EC-no. 231-821-4); sodium metabisulphite (Na ₂ S ₂ O ₅) = Na disulphite or Na pyrosulphite, M = 190.10 (CAS-no. 7681-57-4; EC-no. 231-673-0)
Alkali sulphites are soluble in water setting free active SO ₂ increasingly with decreasing pH. Metabisulphites are more stable to oxidation than bisulphites, which, in turn, show greater stability than sulphites.	
Toxicity data (for SO ₂)	
LC ₀ on inhalation	3000 mg/l (5 min) for humans
LC ₅₀ on inhalation	2250 mg/l (1 h) for rats 3000 mg/l (0.5 h) for mice
Irritant to skin, mucosa and eyes.	
ADI value: 0.7 mg/kg body weight.	
Occupational exposure limit: 5 (2) mg/m ³ (ppm)	
Not classifiable as a human carcinogen.	
Sulphites are GRAS in the USA.	

Antimicrobial effectiveness/applications

SO₂/sulphurous acid is most effective in acid media (pH < 4); the activity decreases from SO₂ → H₂SO₃ → HSO₃⁻ → SO₃²⁻. At pH 3 only 6% of sulphurous acid is available in its effective undissociated state. The anhydride SO₂ is highly reactive and may interact with many constituents of the microbial cell, e.g. SH groups in structural proteins, enzymes, nucleic acids and lipids. Because of its reductive properties SO₂ also acts as an antioxidant.

Due to its properties sulphur dioxide has been used for many centuries mainly for the protection of acidic foodstuffs, especially in the wine industry. However its application is restricted owing to flavour problems occurring at concentrations above 5 mg/kg. It is applied as liquefied gas, a solution in water (H₂SO₃) or the a.m. salts. In the EC list of preservatives which cosmetics may contain inorganic sulphites and hydrogensulphites are mentioned with a maximum concentration of 0.2% expressed as free SO₂. But in finished products it is scarcely used, as these normally do not exhibit the low pH which is required for the antimicrobial effectiveness of sulphur dioxide or sulphurous acid. – Percentage of use in US cosmetic formulations: Sodium sulphite 0.32%; sodium metabisulphite 0.01%; sodium bisulphite 0.26%.

9. Acid esters

Although the microbicides described in this section all belong to the same substance class, namely to the acid esters, their antimicrobial activity is based on different mechanisms of action. The halogen containing esters (9.2.–9.6.) and dimethyl dicarbonate (9.7.) are substances with electrophilic character, a character which enables them to react with nucleophilic groups of the microbial cell and which equips the substances with a wide spectrum of antimicrobial effectiveness. The electrophilic character of the halogenated esters mentioned here is reduced due to the fact that the substances are bearing an activated halogen group in the α -position to an electronegative group (see 17.). Bromo compounds are preferred, because due to the average electronegativity of the bromo atom in comparison to other halogen atoms (for scale of electronegativity values of halogen atoms see Table 83) these compounds are not too stable (not persistent) but also not too reactive (unstable), so that they are preferably used as slimicides not causing waste problems.

Table 83 Relative electronegativity values of halogen atoms according to müller (1951).

Fluoro (F)	4.0
Chloro (Cl)	3.0
Bromo (Br)	2.8
Iodo (I)	2.4

Using these microbicides one has to bear in mind that in accordance with their reactivity these chemicals have skin and mucous membrane irritating properties.

The dicarbonate configuration in dimethyl dicarbonate (DMDC, 9.7.) is responsible not only for the electrophilic character of the chemical but also for its distinguished reactivity (instability), which does not allow use of DMDC as a preservative, but as a powerful cold sterilizing agent.

The phenyl esters of long chain fatty acids (Sections 9.8 and 9.9) are membrane active microbicides; they release phenolic compounds as the active ingredients. The advantage of these esters is that they are easier to handle and to apply than the phenolic compounds they are based on.

Finally it has to be pointed out that the antimicrobial esters of *p*-hydroxy-benzoic acid (*p*-hydroxy-benzoates) are not described in this chapter, because of their high acidity they are listed under 8. Acids.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

Solidification point °C

Density g/ml (20°C)

Vapour pressure hPa (20°C)

Refractive index nD (20°C)

Flash point °C

Upper flammability limit %v/v i.air

Lower flammability limit %v/v i.air

Stability

9. ACID ESTERS

9.1. Ethyl formate

$C_3H_6O_2$

H-COOC₂H₅

74.08

109-94-4

203-721-0

Data base, Jan. 2001

formic acid ethylester, ethyl formic ester

FLUKA

colourless, volatile fluid with an odour similar to arrack

~98

52–55

– 80

0.921

261

1.360

– 19.44

28

16

sensitive to hydrolysis especially in solutions with a pH > 6

Solubility	hardly soluble in H ₂ O; soluble in organic solvents
Toxicity data	
LD ₅₀ oral	1850 mg/kg rat 2075 mg/kg rabbit 1110 mg/kg guinea pig
LD ₅₀ dermal	> 20 mg/kg rabbit
Mildly irritant on the skin; vapours irritate eyes and respiratory tract.	
Occupational exposure limit	300 (100) mg/m ³ (ppm)

Antimicrobial effectiveness/applications

Ethyl formate has to be regarded as a formic acid releasing compound and corresponds in its activity to the quantity of acid set free (see 8.1.1.)

Microbicide group (substance class)	9. ACID ESTERS
Chemical name	9.2. Ethyl bromoacetate
Chemical formula	C ₄ H ₇ BrO ₂
Structural formula	Br-CH ₂ -COOC ₂ H ₅
Molecular mass	167.01
CAS-No.	105-36-2
EC-No.	203-290-2
EPA genotox program 1988	tumorigenic
Synonym/common name	bromoacetic acid ethyl ester, ethoxycarbonylmethyl bromide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless, irritant fluid having an unpleasant smell
Content (%)	~100
Boiling point/range °C (101 kPa)	168–169
Density g/ml (20°C)	1.499
Vapour pressure hPa (20°C)	3.57
Refractive index n _D (20°C)	1.4510
Flash point °C	48
Stability	hydrolysis in aqueous solutions (increasingly with increasing pH values) to hydroxyacetic acid under nucleophilic substitution of the bromo atom
Solubility	sparingly soluble in water, highly soluble in organic solvents, miscible with ethanol
Toxicity data	
LD ₅₀ oral	50 mg/kg rat 100 mg/kg mouse
Highly toxic by inhalation and skin absorption. Severely irritant to skin, mucous membranes and eyes. Tumorigenic.	

Antimicrobial effectiveness/applications

2-Bromo-ethylacetate is especially effective against yeasts, but at higher concentrations also against bacteria, fungi, slime forming micro-organisms and algae. The active ingredient has been used as a non-persistent preservative in drinks, e.g. wine and fruit juices. However, these applications are no longer permitted because of the toxicity and the irritant properties of the compound.

Microbicide group (substance class)	9. ACID ESTERS
Chemical name	9.3. Benzyl bromoacetate
Chemical formula	$C_9H_9BrO_2$
Structural formula	$Br-CH_2-COOCH_2-C_6H_5$
Molecular mass	229.08
CAS-No.	5437-45-6
EC-No.	226-611-4
EPA FIFRA 1988	Pesticide subject to registration or re-registration
Synonym/common name	bromoacetic acid benzyl ester, bromoacetic acid phenylmethyl ester
Supplier	MERCK, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless irritant fluid
Content (%)	~100
Boiling point/range °C (2.9 kPa)	166 – 170
Density g/ml (20°C)	1.46
Refractive index nD (20°C)	1.5436
Flash point °C	> 110
Stability	sensitive to hydrolysis in aqueous alkaline solutions
Solubility	sparingly soluble in water, highly soluble in organic solvents

Toxicity data:

Irritant to skin, mucous membranes, eyes, respiratory tract. May be toxic by inhalation and skin absorption.

Antimicrobial effectiveness/applications

Benzyl bromoacetate acts as an electrophilic active compound; due to its electron attracting power (electronegativity) the bromo atom may be substituted nucleophilically, i.e. by nucleophilic active components of the microbe cell.

On hydrolytic cleavage of the ester group benzyl alcohol (1.4.) is liberated, an membrane active microbicide. These properties equip benzyl bromoacetate with a broad spectrum of activity which covers bacteria, yeasts and fungi. It may be used as a preservative for the in-can protection of water based functional fluids, e.g. paints. However, due to its properties- irritant, moderate stability-the microbicide has been applied to a limited extent only.

Microbicide group (substance class)	9. ACID ESTERS
Chemical name	9.4. 1,2-Bis(bromoacetoxyl) ethane
Chemical formula	$C_6H_8Br_2O_4$
Structural formula	$Br-CH_2-COO-CH_2-CH_2-OOC-CH_2-Br$
Molecular mass	303.94
CAS-No.	3785-34-0
EC-No.	223-250-4
Synonym/common name	ethylene bromoacetate, bromoacetic acid ethenediyl ester
Supplier	DEAD SEA BROMINE GROUP
Chemical and physical properties	
Appearance	almost colourless, irritant fluid
Content (%)	~100
Boiling point/range °C (1,85 kPa)	176.5–177.5
Stability	hydrolysis in water based solutions (increasingly with increase in pH and temperature) to glycol, bromoacetic acid and further to hydroxyacetic acid
Solubility	sparingly soluble in water, highly soluble in alcohols, ether and benzene

Toxicity data

LD ₅₀ oral	> 400 mg/kg rat
LD ₅₀ intraperitoneal	39 mg/kg mouse
LD ₅₀ intravenous	56 mg/kg mouse

Severely irritant to skin, mucosa and eyes.

Ecotoxicity:

Due to its distinct reactivity the microbicide is unstable in the environment (see: Stability) and easily degraded.

Antimicrobial effectiveness/applications

The electrophilic active compound impresses by its activity against slime forming microorganisms. Hence it has been an active ingredient in non-presistant slimicides for use preponderantly in the paper industry.

Microbicide group (substance class)	9. ACID ESTERS
Chemical name	9.5. 1,4-Bis(bromoacetox)-2-butene, (BBAB)
Chemical formula	C ₈ H ₁₀ Br ₂ O ₄
Structural formula	$\text{Br}-\text{CH}_2-\underset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{O}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-\text{O}-\underset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{CH}_2-\text{Br}$
Molecular mass	329.97
CAS-No.	20679-58-7
EC-No.	243-962-9
Synonym/common name	2,3-butylene bromoacetate, bromoacetic acid 2,3-butene-1, 4-diyl ester
Supplier	DEAD SEA BROMINE GROUP, BUCKMANN
Chemical and physical properties	
Appearance	dark brown, oily liquid with an irritant odour
Content (%)	> 95
Boiling point/range °C (6.7 × 10 ⁻⁴ kPa)	135–136
Solidification point °C	< -20
Stability	sensitive to hydrolysis, especially in aqueous systems at pH values > 7 (release of bromoacetic acid and finally butenyl alcohol, hydroxyacetic acid and bromide); half-lives: 8.25 days at pH 5; 6.89 h at pH 7; 4.15 min at pH 9 (Cohen, 1997)
Solubility	practically insoluble in water; soluble in organic solvents, such as acetone, toluene, methylene chloride
Toxicity data (source: DEAD SEA BROMINE GROUP)	
LD ₅₀ oral	191 mg/kg rat 125 mg/kg mouse
LD ₅₀ dermal	983 mg/kg rat
Corrosive to skin, eyes, upper respiratory tract and mucous membranes. – Not mutagenic (Ames test). Not included in NTP 9th Report on carcinogens.	
Ecotoxicity:	
LC ₅₀ for Zebra fish	0.32 mg/l (48 h)
EC ₅₀ for Daphnia magna	0.024 mg/l (24 h)
EC ₅₀ for fresh water algae	0.29 mg/l (96 h)
BBAB is classified as biodegradable.	

Antimicrobial effectiveness/applications

The minimum inhibition concentrations of bis-1,4-(bromoacetoxy)-2-butene for fungi and some species of bacteria are in the range of 20 mg/litre only (see Table 84). The a.i. therefore was successfully introduced as a substitute for the persistent and highly toxic organomercurials (19.) and penta-chlorophenol (7.5.4.) in slimicides for the treatment of industrial water circuits, mainly in the paper industry.

Table 84 Minimum inhibition concentrations (MIC) of bis-1,4-(bromoacetoxy)-2-butene in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	< 20
<i>Botrytis cinerea</i>	20
<i>Chaetomium globosum</i>	< 20
<i>Penicillium glaucum</i>	35
<i>Bacillus subtilis</i>	20
<i>Escherichia coli</i>	200
<i>Staphylococcus aureus</i>	200

Microbicide group (substance class)

Chemical name

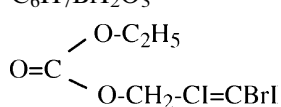
Chemical formula

Structural formula

9. ACID ESTERS

9.6. 1-Bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene

$C_6H_7BrI_2O_3$



Molecular mass

CAS-No.

EC-No.

Synonym/common name

460.84

77352-88-6

unknown

(3-bromo-2,3-diiodopropenyl)-ethyl carbonate, (3-bromo-2,3-diiodoallyl)-ethyl carbonate

Supplier

SANKYO CO.

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Vapour pressure hPa (20°C)

Stability

white crystals with a faint characteristic odour

~100

40

2.4×10^{-5}

limited thermostability at temperatures $> 40^\circ\text{C}$; stable to UV light; hydrolyses in alkaline solutions

Solubility g/l (25°C)

0.119 in H_2O , soluble in organic solvents

Toxicity data

LD₅₀ oral

dermal

LC₅₀ on inhalation (4 h)

641–529 mg/kg rat

2858–2849 mg/kg rat

820–1480 mg/m³ for rats

Moderately irritant to skin and mucosa. Several mutagenicity tests conducted in different biological systems demonstrated lack of genetic effects.

Antimicrobial effectiveness/applications

In view of the minimum inhibition concentrations listed in Table 85 it has to be stated that the electrophilic active microbicide's antimicrobial activity is above all directed against fungi. On cleavage the carbonic acid ester liberates ethanol and 3-bromo-2,3-diiodoallyl alcohol; the latter represents a highly reactive and effective agent as well. The efficacy of (3-bromo-2,3-diiodoallyl)-ethyl carbonate is not influenced by anionic, cationic or non-ionic components. Because of its effectiveness against wood-rotting fungi it has been recommended for use in wood preservatives, and for the protection of plywood incorporating the fungicide into the glue for the production of plywood.

Table 85 Minimum inhibition concentrations (MIC) of (3-bromo-2,3-diiodoallyl)-ethyl carbonate in nutrient agar

Test organisms	MIC (mg/l)
<i>Alternaria alternata</i>	50
<i>Aspergillus niger</i>	20
<i>Aureobasidium pullulans</i>	50
<i>Chaetomium globosum</i>	20
<i>Cladosporium cladosporioides</i>	10
<i>Lentinus tigrinus</i>	20
<i>Penicillium glaucum</i>	15
<i>Sclerophoma pityophila</i>	50
<i>Trichoderma viride</i>	100
<i>Escherichia coli</i>	350
<i>Staphylococcus aureus</i>	150

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Registration

Supplier

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

Solidification point °C

Density g/ml (20°C)

Vapour pressure hPa (20°C)

Viscosity mPas (20°C)

Refractive index nD (20°C)

Flash point °C

Ignition temperature °C

Upper flammability limit %v/v i.air

Lower flammability limit %v/v i.air

Stability

9. ACID ESTERS

9.7. Dimethyl dicarbonate (DMDC)

C₄H₆O₅H₃C-O-CO-O-CO-O-CH₃

134.09

4525-33-1

224-859-8

as food additive permitted for direct addition to food for human consumption—FDA Register 21 CFR Part 172.133. E 242.

BAYER

colourless liquid with a slightly pungent odour

> 99.5

approx. 172 (decomposition)

17

1.25

0.7

2.1

1.3915–1.3925

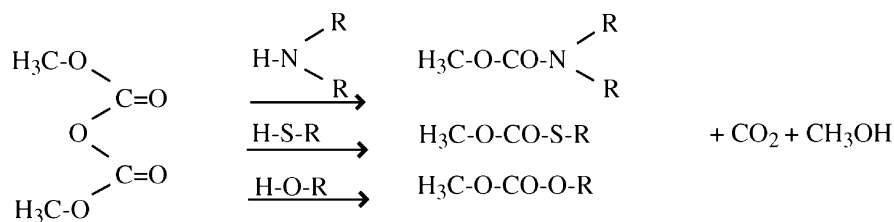
approx. 85

approx. 465

29.9

3

highly reactive carbmethoxylation agent, which means hydrolysis in water to methanol and carbon dioxide, reaction with N–H, S–H and O–H groups according to the following reaction scheme:



Half-life in water

At pH 2.8/10°C: 40 min

pH 2.8/20°C: 15 min

pH 2.8/30°C: 8 min

Solubility

approx. 35 in H₂O; miscible in organic solvents, e.g. ethanol, toluene

Toxicity data

LD ₅₀ oral	497 mg/kg male rat
	335 mg/kg female rat
LD ₅₀ dermal	> 1250 mg/kg rat
LC ₅₀ on inhalation (4 h)	711 mg/m ³ air for rats
DMDC causes severe irritation to skin, eyes, mucous membranes and the respiratory tract.	

Ecotoxicity:

LC ₀ for fish (<i>Leuciscus idus</i>)	50 mg/l (48 h)

Remark: In contact with water DMDC decomposes completely to methanol and carbon dioxide (see: half life).

Antimicrobial effectiveness/applications

The strong reactivity of DMDC is responsible for the antimicrobial action. When checking the microbicidal effectiveness of DMDC one has to bear in mind the short-half life of the chemical in water based media depending on temperature and pH. DMDC kills normal yeasts, mycoderma and fermentive bacteria at relatively low concentrations. At higher concentrations it also destroys other bacteria, wild yeasts and mould producing fungi. Minimal lethal concentrations of DMDC for a great number of individual microbe species are listed in Table 86.

The killing effect of DMDC bases on its irreversible reaction with nucleophilic components of microbe cells. In consequence DMDC destroys high cell numbers only at higher concentrations. The lethal concentrations in Table 86 were determined as follows: the microbe species concerned was introduced into an uncarbonated acidic

Table 86 Minimum lethal concentrations (MIC) of DMDC (mg/litre)

Yeasts	
<i>Saccharomyces carlsbergensis</i> (non-flocculating yeast)	100
<i>Saccharomyces carlsbergensis</i> (flocculating yeast)	60
<i>Saccharomyces diastaticus</i>	200
<i>Saccharomyces oviformis</i>	100
<i>Saccharomyces bailii</i>	120
<i>Saccharomyces cerevisiae</i>	40
<i>Saccharomyces uvarum</i>	30
<i>Saccharomyces pastorianus</i>	100
<i>Saccharomyces apiculatus</i>	60
<i>Saccharomyces globosum</i>	40
<i>Zygosaccharomyces priorianus</i>	75
<i>Rhodotorula mucilaginosa</i>	50
<i>Rhodotorula glutinosa</i>	40
<i>Rhodotorula rubra</i>	200
<i>Candida krusei</i>	200
<i>Pichia membranefaciens</i>	40
<i>Pichia farinosa</i>	100
<i>Torulopsis candida</i>	100
<i>Torulopsis versatilis</i>	100
<i>Torulopsis stellata</i>	65
<i>Torula utilis</i>	240
<i>Endomyces lactis</i>	60
<i>Kloeckera apiculata</i>	40
<i>Hansenula anomala</i>	50
Bacteria	
<i>Acetobacter pastorianum</i>	80
<i>Acetobacter xylinum</i>	300
<i>Escherichia coli</i>	400
<i>Staphylococcus aureus</i>	100
<i>Pseudomonas aeruginosa</i>	100
<i>Lactobacterium buchneri</i>	40
<i>Lactobacillus pastorianus</i>	300
<i>Lactobacillus brevis</i>	200
<i>Pediococcus cerevisiae</i>	300
Moulds	
<i>Penicillium glaucum</i>	200
<i>Byssoschlamys fulva</i>	100
<i>Botrytis cinerea</i>	100
<i>Mucor racemosus</i>	500
<i>Fusarium oxysporum</i>	100

drink (approx. pH 3) to give a viable cell count of 500 per ml; the effect of the treatment with DMDC was determined after the drink had been stored for 3 weeks at 28°C.

Although DMDC is highly effective, it cannot, because of its short half-life, be used as a preservative in water based media, when there is a risk of recontamination after the addition of DMDC. However, DMDC has found application for the cold sterilization of soft drinks and wine, and for the degermination of water, which is used for the production of drinks, cosmetics and pharmaceuticals. Once DMDC has decomposed there is no further sterilizing effect. It should therefore not be added until shortly before the drink is put into bottles or other containers and tightly closed. It has to be regarded as an important advantage that DMDC is not a persistent preservative and that its application does not influence either taste or quality of drinks. Addition rates range between 10 and 20 ml DMDC per 100 litre drink. But before DMDC is added the number of viable cells in the drink has to be reduced to approx. 500 per ml by filtration or flash pasteurization; the latter also inactivates enzymes which may decompose pectin. It is also recommendable to cool the drink before the addition of DMDC, preferably to 10–15°C; otherwise DMDC decomposes too fast not leaving time for sufficient antimicrobial action.

Microbicide group (substance class)	9. ACID ESTERS
Chemical name	9.8. Glyceryl monolaurate (α - and β -form)
Chemical formula	$C_{15}H_{30}O_4$
Structural formula	$H_3C-(CH_2)_{10}-COO-CH_2-CH(OH)-CH_2OH$ (α -form)
Molecular mass	274.41
CAS-No.	27215-38-9
EC-No.	unknown
FDA Approval	for food use as an emulsifier (21 CFR GRAS 182.4505)
Synonym/common name	Lauricidin, 1- and 2-lauroylglycerol, n-dodecanoic acid monoglyceride
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	powder or pastelike, off-white solid
Content (%)	> 90
Melting point °C	55–56
Stability	very stable under normal conditions; with increasing storage time the more stable α -form reaches values of 90–95%; unaffected in the pH range 3.5–8; on hydrolyses glycerine and Lauric acid (8.1.13.) are liberated
Solubility g/l (25°C)	< 1 in H_2O , 2500 in methanol, 800 in ethanol, 600 in isopropanol, 45 in propylene glycol, 2 in glycerine and mineral oil
Toxicity data (source: Kabara, 1984)	
LD ₅₀ oral	> 25 g/kg mouse
Only moderately irritant to skin and eyes.	Classified as a mild sensitizer (grade II; guinea pig test according to Magnusson-Kligman).

Antimicrobial effectiveness/application (Kabara, 1984)

Lauricidin can be characterized as a nonionic emulsifier with antimicrobial properties. There is no difference in the antimicrobial activity of both isomeric monoglycerides. Lauricidin's antibacterial activity is restricted to gram-positive bacteria (MIC ~5000 mg/l); MIC's for Gram-negative bacteria are beyond 10000 mg/l. However the activity against molds and yeasts and also against lipid-coated viruses is remarkable. It is recommended to applicate Lauricidin in combination with other microbicides (Parabens, etc.) to achieve a sufficiently broad spectrum of effectiveness, e.g. for the in-can protection of cosmetic and pharmaceutical products. Addition rates 0.5–1%; optimum pH 6–7.5.

Microbicide group (substance class)

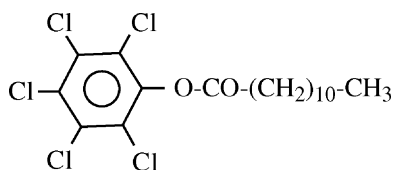
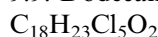
Chemical name

Chemical formula

Structural formula

9. ACID ESTERS

9.9. Dodecanoic acid pentachlorophenyl ester



Molecular mass

448.65

CAS-No.

3772-94-9

EC-No.

308-706-3

Synonym/common name

pentachlorophenyl laurate

Chemical and physical properties

Appearance

oily, brown, odourless liquid

Content (%)

approx. 100

Density g/ml (20°C)

1.25

Stability

non-volatile, unaffected by dilute acids or alkalis,
hydrolysis by high concentrations of alkali or by enzymatic
action, sensitive to photochemical breakdown

Solubility

practically insoluble in water and alcohols, soluble in all
portions of acetone, methyl ethyl ketone, trichloroethylene,
toluene, white spirit, oils, fats and waxes

Toxicity data:

Pentachlorophenyl laurate (PCPL) is, as long as pentachlorophenol (7.5.4. = PCP) is not liberated, of low toxicity and good skin compatibility, however the ester may release approx. 57% pentachlorophenol the toxicity data of which are listed under 7.5.4.

Antimicrobial effectiveness/applications

As can be seen from Table 87 the intact PCPL does not exhibit significant antimicrobial activity, especially not in comparison to PCP. Nevertheless PCPL has had considerable use as a microbicide for rot- and mold-proofing of various types of materials, mainly textiles, ropes and cordage (addition rates approx. 2% calculated on the weight of material to be protected), as in fact the active ingredient is PCP which is set free by enzymatic ester cleavage. In consequence PCPL is under pressure for substitution as is PCP and the application of PCPL is indeed in decline.

Table 87 Minimum inhibition concentrations (MIC) of PCPL and PCP in nutrient agar

Test organism	MIC (mg/litre)	
	PCPL	PCP
<i>Aspergillus niger</i>	> 1000	50
<i>Chaetomium globosum</i>	> 1000	20
<i>Penicillium glaucum</i>	> 1000	50
<i>Escherichia coli</i>	> 2500	500
<i>Staphylococcus aureus</i>	750	10

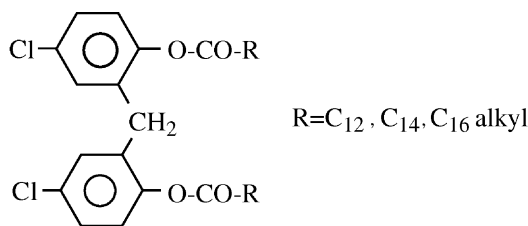
Microbicide group (substance class)

9. ACID ESTERS

Chemical name

9.10. Fatty acid esters (mix.) of 5,5'-dichloro-2,2'-
dihydroxydiphenylmethane (Deiner, 1983)

Structural formula



Supplier

PFERSEE CHEM.

Chemical and physical properties

Appearance

oily, brown liquid of high viscosity

Content (%)

70 Lauric acid ester, 20 Myristic acid ester, 10 Palmitic acid ester

Stability

non-volatile, heat resistant ($> 200^{\circ}\text{C}$), hydrolyses in alkaline solutions

Solubility

insoluble in water, highly soluble in organic solvents, preferably in non-polar solvents

Toxicity data

As the active ingredient of the mixture of esters is Dichlorophen (7.7.3., CAS-no. 97-23-4, EC-no. 292-567-1) which is liberated through enzymatic ester cleavage for antimicrobial action, one has to note the toxicity data of Dichlorophen.

Antimicrobial effectiveness/applications

The mixture of Dichlorophen fatty acid esters develops antimicrobial activity by the reconstitution of Dichlorophen (7.7.3.) through hydrolysis. The most important advantage of the ester mixture is the possibility of transferring it easily into stable emulsions which can be used for the impregnation of textile material together with water repellents without disturbing the effect of the water repellents. The application of Dichlorophen itself is fraught with difficulties. Stable emulsions of Dichlorophen for dilution with water are not available. Alkaline solutions of Dichlorophen are easy to apply on textile material by impregnation, but the alkali salts of Dichlorophen are not compatible with most of the water repellents. The application of Dichlorophen in solutions in organic solvents is not the solution to the problem, as the use of organic solvents is disliked in the textile industry.

Microbicide group (substance class)

9. ACID ESTERS

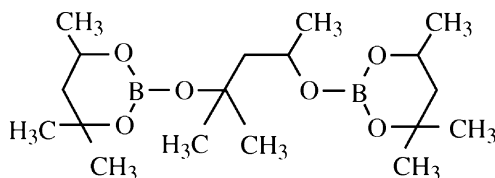
Chemical name

9.11. 2,2'-[(1,1,3-trimethyl-1,3-propanediyl)bis(oxy)]bis[4,4,6-trimethyl-1,2,3-dioxaborinane]

Chemical formula

 $C_{18}H_{36}B_2O_6$

Structural formula



Molecular mass

370.11

CAS-No.

100-89-0

EC-No.

200-899-0

Synonym/common name

2,2'-(2-methylpentane-2,5-dioxy)bis(4,4,6-trimethyl-1,2,3-dioxaborinane), trihexylene glycol baborate

Supplier

RHONE-POULENC

Chemical and physical properties

Appearance

clear, colourless to pale yellow easy pourable liquid

Content (%)

~100 (boron content 5.6–5.8; equivalent to 32.0–33.2% boric acid)

Boiling point/range $^{\circ}\text{C}$ (101 kPa)

314–326 (143–149 at 0.267 kPa)

Density g/ml (20°C)	0.98
Refractive index nD (18.5)	1.4408
Flash point °C	175 (closed cup)
Stability	hydrolyzes in contact with water, even when exposed to atmospheric moisture
Solubility	soluble in all proportions in white spirit, kerosene, carbon tetrachloride, benzene, toluene, xylene, petroleum ether
Toxicity data (source: PHONE-POULENC)	
LD ₅₀ oral	> 4000 mg/kg rat
dermal	> 2000 mg/kg rat
Moderate irritant to skin and mucosa; not a skin sensitizer.	

Antimicrobial effectiveness/applications

Trihexylene glycol baborate can be regarded as a boric acid (8.2.1.) releasing compound. It has been developed for the remedial (preservative) treatment of timber. After penetration in situ the hydrolysis to boric acid occurs providing fungicidal and insecticidal properties. For boric acid on *Pinus sylvestris* sapwood the following toxic limits have been established:

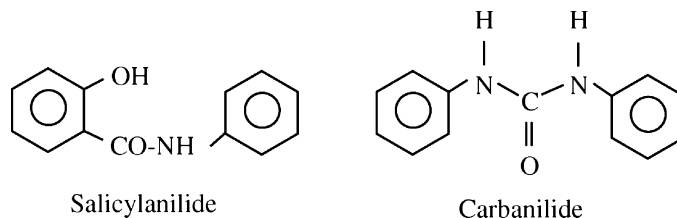
for <i>Coniophora puteana</i>	0.43–0.65 kg/m ³
For <i>Poria xantha</i>	0.08–0.20 kg/m ³
(Forest Products Research Laboratory, Princes Risborough, 1968).	

The baborate is used in solvent based wood preservatives. It is also effective on white rots such as *Polystictus versicolor*. Minimum recommended concentration for in-situ applications: 3.2%.

10 Amides

Carboxylic acid amides do not generally belong to the substances with antimicrobial effect. Toxophoric groups or toxophoric structural elements have to be introduced to obtain antimicrobially active aliphatic carboxylic acid amides. This possibility is exemplified by amides which in 2-position to the electronegative carboxylamide group possess a halogen atom, thus ranking among the electrophilic active microbicides which are described under '17. Compounds with Activated Halogen Atoms'. The addition of formaldehyde (2.1.) to such halogenated amides leads to antimicrobially effective N-hydroxymethyl amides, whose special feature is the presence of two toxophoric groups: an activated halogen atom and an activated hydroxymethyl group. Being formaldehyde releasing compounds, they are treated under '3.4. Amide-Formaldehyde-Reaction-Products', as well as N-hydroxymethyl diamides of carbonic acid = N-hydroxymethyl ureas (3.4.3.).

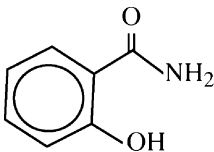
Salicylanilides (2-hydroxybenzanilides), long chain N-alkyl-salicylamides and carbanilides (urea derivatives) belong to the amides with antimicrobial efficacy, too. They are membrane-active substances, i.e. very small concentrations suffice to achieve microbistatic effects whereas microbicidal effects call for much higher addition rates.



As in the case of the membrane-active phenol derivatives (7.) the halogenation of salicylanilides or carbanilides increases the antimicrobial efficacy. The best results are obtained by means of di- to penta-chlorination or bromination, the halogen atoms being more or less evenly distributed on the two phenyl rings. On the other hand halogenated salicyl anilides have photosensitizing properties, which has reduced their practical importance.

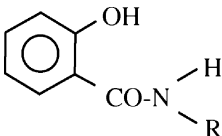
Also haloalkylthio amides are well-known microbicides; they are electrophilic active agents disposing of an activated N-S bond. Their role as an important class of microbicides is described separately under 16.

For sake of completeness it should be mentioned a carboxylic acid hydrazide, namely pyridine-4-carboxylic acid hydrazide (isonicotinic acid hydrazide, Isoniazide), a pyridine derivative which is appropriately described under 13. 'Pyridine Derivatives and Related Compounds' (see 13.2.).

Microbicide group (substance class)	10. AMIDES
Chemical name	10.1. Salicylamide
Chemical formula	$C_7H_7NO_2$
Structural formula	
Molecular mass	137.13
CAS-No.	65-45-2
EC-No.	200-609-3
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	2-hydroxybenzoic acid amide, o-hydroxybenzamide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to slight yellow crystalline powder
Content (%)	min. 99
Boiling point/range °C (1.9 kPa)	182
Melting point °C	142
Density g/ml	1.175
Stability	stable under normal conditions
Solubility	soluble in alcohols, acetone, ether, alkaline solutions and in boiling water
Toxicity data	
LD ₅₀ oral	980 mg/kg rat 300 mg/kg mouse 3200 mg/kg rabbit
LD ₅₀ intraperitoneal	600 mg/kg rat 180 mg/kg mouse
LD ₅₀ subcutaneous	300 mg/kg mouse
LD ₅₀ intravenous	313 mg/kg mouse
100 mg (24 h) were moderately irritant to rabbit eyes.	
Salicylamide is considered as possibly teratogenic.	

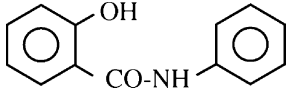
Antimicrobial effectiveness/application

Remarkable is the fungicidal activity of salicylamide which led to its application in soaps, ointments and powders. However, as an active ingredient in formulations for the protection of materials salicylamide has no importance.

Microbicide group (substance class)	10. AMIDES
Chemical name	10.2. N-Alkylsalicylamides
Structural formula	 R = alkyl (C₄–C₁₄)
Synonym	2-hydroxy-N-alkylbenzamides
Chemical name	10.2.1. N-Butylsalicylamide
Chemical formula	$C_{11}H_{15}NO_2$
Molecular mass	193.25
CAS-No.	57271-91-7
Appearance	viscous oil

Chemical name	10.2.2. N-Hexylsalicylamide
Chemical formula	$C_{13}H_{19}NO_2$
Molecular mass	221.30
CAS-No.	67520-12-1
Appearance	solid, mp 42–43°C
Chemical name	10.2.3. N-Octylsalicylamide
Chemical formula	$C_{15}H_{23}NO_2$
Molecular mass	249.36
CAS-No.	109972-90-9
Appearance	solid, mp 44–46°C
Chemical name	10.2.4. N-Decylsalicylamide
Chemical formula	$C_{17}H_{27}NO_2$
Molecular mass	277.41
CAS-No.	116311-05-8
Appearance	solid, mp 59–60°C
Chemical name	10.2.5. N-Dodecylsalicylamide
Chemical formula	$C_{19}H_{31}NO_2$
Molecular mass	305.46
CAS-No.	10586-70-6
Appearance	solid, mp 64–70°C
Chemical name	10.2.6. N-Tetradecylsalicylamide
Chemical formula	$C_{21}H_{35}NO_2$
Molecular mass	333.52
CAS-No.	109972-89-6
Appearance	solid, mp 74–77°C

The long chain *N*-alkylsalicylamides are moderately soluble in water and fairly soluble in organic solvents. Their antimicrobial effectiveness is described by Leinen *et al.* (1988). Among the salicylamides mentioned here the *N*-octyl and *N*-decyl derivatives are the most effective ones. In concentrations of 2.5–10 mg/litre these derivatives inhibit the proliferation of Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus mutans* or *Actinomyces viscosus*. *N*-alkylsalicylamides generally act at first as microbistats. Because of their selective activity they may be used as active ingredients in deodorant formulations, as it is known that the odour producing bacteria on the skin mainly belong to the group of Gram-positive organisms. *N*-alkylsalicylamides are also useful for incorporation into antimicrobial preparations as they strongly increase the activity of the preparations against cocci.

Microbicide group (substance class)	10. AMIDES
Chemical name	10.3. Salicylanilide
Chemical formula	$C_{13}H_{11}NO_3$
Structural formula	
Molecular mass	213.24
CAS-No.	87-17-2
EC-No.	201-727-8
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	2-hydroxybenzanilide, N-phenylsalicylamide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white, odourless crystals
Content (%)	~100
Melting point °C	136–138

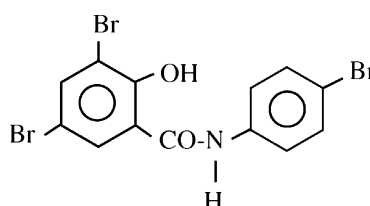
Stability	relatively heat resistant, not volatile
Solubility	very limited solubility in water; forms water soluble alkali salts; soluble in organic solvents
Toxicity data	
LD ₅₀ oral	2400 mg/kg mouse
LD ₅₀ intraperitoneal	> 500 mg/kg mouse
Irritant to skin, eyes and mucous membranes.	

Antimicrobial effectiveness/application

Salicylanilide is especially active against fungi. As it is a practically odourless compound which does not cause coloration and is not hazardous in application, it has been used for the protection of textiles, leather, paper, plastic, paints, adhesives, etc. However, the compound is susceptible to leaching and therefore does not perform satisfactorily on material exposed outdoors. Effective addition rates are high, e.g. up to 10% in paints. Salicylanilide therefore has been widely substituted by more active and more economic microbicides. The sodium salt of salicylanilide may be used in fungicidal wall washes.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

10. AMIDES
10.4. 3,4',5-Tribromosalicylanilide
C₁₃H₈Br₃NO₂



Molecular mass
CAS-No.
EC-No.
Synonym/common name

449.96
87-10-5
201-723-6
Tribromosalan (TBS)

Chemical and physical properties

Appearance
Content (%)
Melting point °C
Stability

white, odourless crystals
~100
227–228
light and heat resistant; not compatible with proteins, compatible with anionic and non-ionic detergents
virtually insoluble in H₂O and paraffins; soluble in aqueous alkalis, hot acetone, dimethylformamide

Solubility

Toxicity data

LD₅₀ oral
Only moderately irritant to skin and mucosa.
Strong skin sensitizing agent.

570 mg/kg rat

Antimicrobial effectiveness/application

TBS disposes of excellent bactericidal properties. It was mainly used as an active ingredient in deodorizing soaps; addition rate max. 1%. However, its application generally has been largely stopped because of the sensitizing activity.

Microbicide group (substance class)

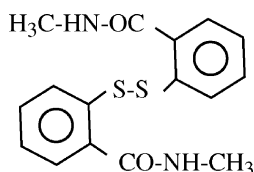
Chemical name

Chemical formula

Structural formula

10. AMIDES

10.5. Dithio-2,2'-bis(benzmethylamide)

 $C_{16}H_{16}N_2O_2S_2$ 

Molecular mass

CAS-No.

EC-No.

EPA TSCA USA/MITI Japan

Synonym/common name

Supplier

332.45

2527-58-4

219-768-5

Chemical Inventory

dithio-2,2'-bis(benzoic acid methyl amide)

AVECIA

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Vapour pressure hPa (20°C)

Stability

wet, off-white fawn powder with a bland odour

~100

~214

 $< 4 \times 10^{-13}$

hydrolyses in alkaline media to the microbicide N-methyl-benzisothiazolinone (15.); excellent heat stability; incompatible with oxidizing and reducing agents, such as persalts, sulphites

Solubility g/l

0.20 in H_2O , 1.0 in methyl ethyl ketone, 0.02 in toluene, 0.01 in white spirit

Toxicity data (source: AVECIA)

LD₅₀ oral

dermal

> 2000 mg/kg rat

> 2000 mg/kg rat

Irritant to skin and eyes. May cause sensitization by skin contact.

Ecotoxicity:

LC₅₀ for Rainbow troutEC₅₀ for Daphnia magnaEbC₅₀ for green algaeErC₅₀

1.2 mg/l (96 h)

0.044 mg/l (48 h)

0.26 mg/l (72 h)

0.61 mg/l (72 h)

Testparameter: b = biomass; r = growth rate.

Tests with activated sludge organisms show that the inhibition of aerobic waste water bacteria is unlikely.

The microbicide is classified as not readily biodegradable.

Antimicrobial effectiveness/applications

As is shown by the MIC in Table 88., dithio-2,2'-bis(benzmethylamide)'s spectrum of effectiveness covers in particular fungi, but yeasts and bacteria, too. This and the solubility properties of the microbicide make it suitable for anti-fungal protection of dry films (paints, adhesives, sealants) and for wet state preservation applications. The addition rates of a formulation containing 25% a. i. move between 0.05–0.20% for the in-can/in-tank preservation of aqueous products and 0.5–2.0% for dry film applications. The agent is active over pH range 4–12.

Table 88 Minimum inhibition concentrations (MIC) of dithio-2,2'-bis (benzomethylamide) (25%) (Source: AVECIA)

Test organismen	MIC (mg/l)
Bacteria	
<i>Bacillus subtilis</i>	40
<i>Enterobacter cloacae</i>	50
<i>Escherichia coli</i>	80
<i>Proteus vulgaris</i>	50
<i>Pseudomonas aeruginosa</i>	500
<i>Pseudomonas putida</i>	30
<i>Rhodopseudomonas capsulata</i>	150
<i>Streptococcus aureus</i>	40
<i>Streptococcus faecalis</i>	40
Yeasts	
<i>Rhodotorula rubra</i>	75
<i>Saccharomyces cerevisiae</i> (turbidans)	15
Fungi	
<i>Aspergillus flavus</i>	15
<i>Aspergillus niger</i>	60
<i>Aspergillus oryzae</i>	15
<i>Aspergillus versicolor</i>	50
<i>Aureobasidium pullulans</i>	30
<i>Chaetomium globosum</i>	30
<i>Cladosporium sphaerospermum</i>	3
<i>Fusarium solani</i>	75
<i>Penicillium expansum</i>	75
<i>Penicillium notatum</i>	25
<i>Penicillium rubrum</i>	15
<i>Trichoderma viride</i>	3

Microbicide group (substance class)

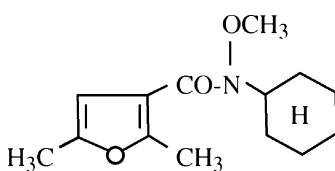
Chemical name

Chemical formula

Structural formula

10. AMIDES

10.6. N-Cylohexyl-N-methoxy-2,5-dimethyl-3-furan carboxamide

 $C_{14}H_{21}NO_3$ 

Molecular mass

251.33

CAS-No.

60568-05-0

EC-No.

262-302-0

Synonym/common name

N-cylohexyl-N-methoxy-2,5-dimethyl-3-furamide, Furmecyclox

Chemical and physical properties

Appearance

yellow, slightly viscous fluid

Content (%)

~100

Boiling point/range °C (0.07 kPa)

135–140

Solidification point °C

~30

Density g/ml (20°C)

1.081

Vapour pressure hPa (20°C)

 9.6×10^{-5}

Stability

hydrolyzes in strong acids and alkalis

Solubility g/l

< 1 in H₂O; highly soluble in organic solvents

Toxicity data

LD₅₀ oral

3780 mg/kg rat

~3500 mg/kg rabbit

dermal

> 5000 mg/kg rat

LC₅₀ inhalative5600 mg/m³ air (4 h) for rats

Antimicrobial effectiveness/application

Furmecyclox is a fungicide which is especially active against wood-rotting fungi, e.g. *Coniophora puteana*, *Coriolus versicolor*, *Lentinus lepideus* and *Lenzites abietina*. It has been used therefore as an active ingredient in preservatives for the protection of wood, generally in combination with other fungicides which are active against wood staining fungi, e.g. *Aureobasidium pullulans*. However, in the meantime the application of Furmecyclox has been discontinued and azole fungicides (14.) have developed to important substitutes.

Microbicide group (substance class)

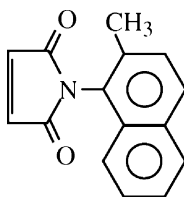
Chemical name

Chemical formula

Structural formula

10. AMIDES

10.7. N-(2-methylnaphthyl)maleinimide

 $C_{15}H_{11}NO_2$ 

Molecular mass

237.26

CAS-No.

70017-56-0

EC-No.

unknown

Synonym/common name

N-(2-methylnaphthyl)pyrrol-2,5-dione

Chemical and physical properties

Appearance

white powder

Content (%)

~100

Melting point °C

163–165

Density g/ml

1.25–1.28

Stability

heat resistant (> 190°C)

Solubility g/l (25°C)

0.025 in H₂O, 4 in ethanol, 2 in isopropanol, 3 in propylene glycol, ~180 in dimethylsulphoxide, ~100 in acetone, ~60 in dimethylphthalate, 20 in toluene, 50 in tetrahydrofuran

Toxicity data

LD₅₀ oral

> 7000 mg/kg rat

dermal

> 7000 mg/kg rat

Acute inhalation: lethal concentration > 10.5 mg/l

Slightly irritant to the skin, moderately irritant to mucous membranes; did not produce any sensitization in the guinea pig test.

No indications of mutagenic potential in the Ames test.

Not teratogenic in tests with rats and rabbits.

Ecotoxicity:

LC₅₀ for trouts

34 ppb

EC₅₀ for *Daphnia magna*

2.6 ppm

Antimicrobial effectiveness/applications

N-(2-methylnaphthyl)maleinimide has been found to be a microbicide capable of protecting fabrics, plastics, paints, etc., from fungal and bacterial attack (Becker & Gurnee, 1979). As can be seen from the minimum inhibition concentrations in Table 89, it is, however, particularly effective against fungi and yeasts. This activity of the product in combination with its heat stability and low water solubility makes the agent especially recommendable for the antimicrobial treatment of plastics.

Table 89 Minimum inhibition concentrations (MIC) of *N*-(2-methylnaphthyl)maleinimide in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	2
<i>Stv. reticulum</i> (Pink stain)	2–5
Brewer's yeast	10–25
<i>Candida guilliermondii</i>	1–10
<i>Candida lipolytica</i>	1–10
<i>Escherichia coli</i>	100
<i>Klebsiella pneumoniae</i>	20
<i>Pseudomonas aeruginosa</i>	1000
<i>Staphylococcus aureus</i>	20
<i>Streptococcus faecalis</i>	500–1000

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

USA

Japan

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Bulk density g/l

Vapour pressure hPa (50°C)

Flash point °C

Log POW

pH value

Stability

Solubility g/l (20–25°C)

Toxicity data (source. BAYER)

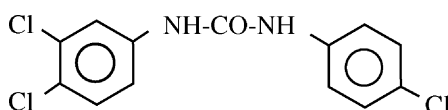
LD₅₀ oral

intraperitoneal

Non-irritant on skin and eyes (tests with rabbits).

No sensitising effect in the guinea pig test according to Magnusson-Kligman.

10. AMIDES

10.8. *N*-(4-chlorophenyl)-*N'*-(3,4-dichloro-phenyl)ureaC₁₃H₉Cl₃N₂O

315.59

101-20-2

202-924-1; EEC-no. 23

CTFA listed

listed in "The Principles of Cosmetic Licensing"

3,4,4'-trichlorocarbanilide (TCC), Triclocarban

BAYER, CLARIANT-NIPA

white crystalline powder with a faint odour

min. 97

250–255 (decomposition)

~650

< 1

> 300

4.2

7 in aqueous suspension

a special merit of Triclocarban, the reaction product of 3,4-dichloroaniline and 4-chlorophenylisocyanate, is its stability

0.11 × 10⁻³ in H₂O, 10 in ethanol, 10 in isopropanol, 48 in dipropylene glycol, 155 in PEG-12, 120 in PEG-8 laurate, 100 in fatty alcohol poly glycol ether

> 2000 mg/kg rat

> 5000 mg/kg mouse

approx. 2100 mg/kg mouse

Ecotoxicity:

According to a test in shaken bottle with adapted bacteria TCC is classified as easy biodegradable (above 90%).

EC ₅₀ for activated sludge organisms	> 10000 mg/l
EC ₅₀ for <i>Daphnia magna</i>	0.015 mg/l (24 h)
EC ₅₀ for algae (<i>Scenedesmus</i> subs.)	~0.025 mg/l (72 h)
LC ₀ for fish (<i>Brachydanio rerio</i>)	> 1000 mg/l (96 h)

Antimicrobial effectiveness/applications

The MIC presented in Table 90 for a broad range of test organisms give a survey of TCC's spectrum of effectiveness and demonstrate that TCC primarily inhibits the growth of Gram-positive bacteria. Typical for the membrane-active compound is that microbicidal activity results only at concentrations being many times the minimum inhibition concentrations. Remarkable is also that TCC unleashes its effect very quickly (within a few minutes). TCC is incompatible with nonionics (Tween 80), phosphatides and protein, but compatible with anionic and cationic tensides. The optimum pH range is 4–8. Worth mentioning is also that TCC is light-stable and does not impair neither the colour nor the odour of products containing it. A preferred active ingredient is Triclocarban in bacteriostatic soaps and other deodorant products such as antiperspirants, shampoos, skin creams and shaving foams. The compound has an affinity to the skin which means that a longer lasting antimicrobial effect is achieved by using a product containing TCC. Addition rates move between 0.2–0.5%. In the EC list of preservatives which cosmetic products may contain TCC is listed with a maximum permissible concentration of 0.2%, however, with the instruction that it can also be used in other concentrations and for different purposes than for the preservation of cosmetic agents, provided that the other purpose is evident from the labeling of the product.—Percentage of use in US cosmetic formulations: 0.02%.

Table 90 Minimum inhibition concentrations (MIC) of Triclocarban in nutrient agar (Source: Bayer)

Microorganisms	Mg/l
Mould fungi	
<i>Fomes annuus</i>	100
<i>Trichophyton gypseum</i>	500
<i>Trichophyton inguinale</i>	500
<i>Chaetomium globosum</i>	100
<i>Memnoniella echinata</i>	100
<i>Microsporum canis</i> CBS 38564	1000–2000
<i>Rhizoctinia solani</i>	100
<i>Trichophyton mentagrophytes</i> CBS 26379	1000
<i>Trichophyton rubrum</i> DSM 4167	2000
Bacteria	
<i>Bacillus subtilis</i> ATCC 11774	35–50
<i>Corynebacterium ammoniagenes</i> ATCC 6871	5
<i>Enterococcus faecalis</i> ATCC 19433	50
<i>Escherichia coli</i> EHEC DSM 8579	1000
<i>Lactobacillus fermentum</i> ATCC 14931	50
<i>Lactobacillus plantarum</i> ATCC 8014	50
<i>Legionella pneumophila</i> ATCC 33152	< 10
<i>Listeria monocytogenes</i> DSM 20600	< 10
<i>Micrococcus luteus</i> ATCC 7468/4698	5–10
<i>Mycobacterium phlei</i> ATCC 11758	< 0.05
<i>Mycobacterium terrae</i> DSM 43227	10–500
<i>Propionibacterium acnes</i> DSM 20458	10–100
<i>Salmonella choleraesuis</i> DSM 4224	1000
<i>Staphylococcus aureus</i> ATCC 6538/29213/33591	10–50
<i>Staphylococcus aureus</i> MRSA DSM 2569	< 10
<i>Staphylococcus aureus</i> MRSA clinical isolate	< 10
<i>Staphylococcus epidermidis</i> ATCC 12228	10
<i>Staphylococcus haemolyticus</i> ATCC 29970	50
<i>Staphylococcus saprophyticus</i> ATCC 15305	200–500
<i>Staphylococcus warneri</i> ATCC 155	50
<i>Streptococcus agalactiae</i> ATCC 13813	< 0.05
<i>Streptococcus pyogenes</i> ATCC 21059/12344	< 0.05

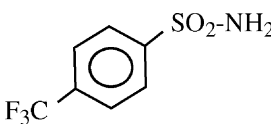
Microbicide group (substance class)	10. AMIDES
Chemical name	10.9. N'-(3,4-dichlorophenyl)-N,N-dimethylurea
Chemical formula	C ₉ H ₁₀ Cl ₂ N ₂ O
Structural formula	
Molecular mass	233.1
CAS-No.	330-54-1
EC-No.	206-354-4
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	3-(3,4-dichlorophenyl)-1,1-dimethylurea, Diuron
Supplier	BAYER, THOR
Chemical and physical properties	
Appearance	white to off white powder with an amine-like odour
Content (%)	min 97
Melting point °C	154–159
Bulk density g/l	450
Vapour pressure hPa (20°C)	2.3×10^{-9} (at 25°C: 6.0×10^{-9})
Ignition temperature °C	approx. 400
Log POW	2.71
pH value	~5.8 in aqueous suspension
Stability	stable under normal conditions; thermal decomposition begins at 200°C; stable in the presence of light and over pH range 3–10 in aqueous systems
Solubility g/l (20°C)	0.035 in H ₂ O, 62 in butyl glycol, 50 in acetone, 1.2 in xylene
Toxicity data (source: BAYER)	
LD ₅₀ oral	approx. 4150 mg/kg rat
dermal	> 5000 g/kg rat
Diuron is not irritant to skin and mucosa. No sensitization was observed in the guinea pig test.	
Ecotoxicity:	
Acute fishtoxicity	
LC ₅₀ on Cyprinus carpio	3.2 mg/l (48 h)
Lepomis macrochirus	7.4 mg/l (48 h)
Poecilia reticulata	25 mg/l (96 h)
Onchorynchus mykiss	14.7 mg/l (96 h)
EC ₅₀ for Daphnia magna	1.4 mg/l (48 h)
EC ₅₀ for algae (Scenedesmus subs.)	0.022 mg/l (96 h)
The activity of waste water bacteria is inhibited (50%) by 3080 mg/l.	
Abiotic degradation: half-life	~5 years at pH 4/22°C
	~6 years at pH 7/22°C
	> 1 year at pH 9/22°C

Antimicrobial effectiveness/applications

Diuron is a broad spectrum algicide which is highly effective against both sea-water and freshwater algae by interrupting the photosynthetic electron transport. There is a big difference between the minimum algistatic concentrations of Diuron (≤ 1 mg/litre) and its minimum algicidal concentrations (100 mg/litre), indicating that the compound acts as an inhibitor of photosynthesis. Its favourable properties – non-volatile, low solubility in water, good toxicological properties, low ecotoxicity – make Diuron ideal for use as an algicide in masonry paints and lately in anti-fouling coatings. Unlike organotin compounds, it is light stable and, more importantly, it has low tolerable ecotoxicity. Although the antimicrobial effect of Diuron covers some species of mould fungus, the overall fungicidal activity of the compound is not very strong. As coatings for exterior applications are subjected to both algal attack

and fungal infestation, an effective and suitable fungicide must also be added to the coating together with Diuron, unless one does not prefer to use a microbicide which has both a fungicidal and algicidal effect.

The minimum application concentrations of Diuron (calculated on total paint weight) move between 0.2–0.5% in masonry paints and 0.05–0.3% in plasters. The concentration in marine antifouling coatings is usually between 2 and 4%.

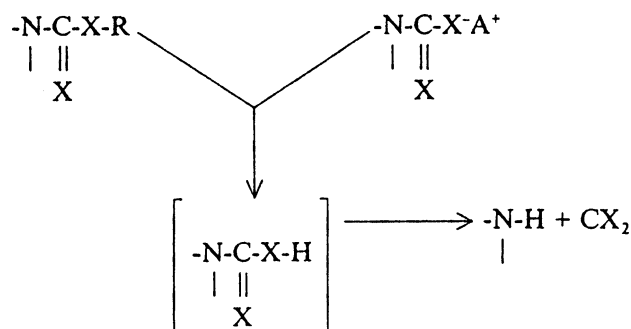
Microbicide group (substance class)	10. AMIDES
Chemical name	10.10. 4-Trifluoromethylphenylsulfonic acid amide
Chemical formula	$C_7H_6F_3NO_2S$
Structural formula	
Molecular mass	225.19
CAS-No.	830-43-3
EC-No.	unknown
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	~100
Melting point °C	181–183
Stability	stable in slightly acid or alkaline media
Solubility	poor solubility in water, soluble in aqueous alcohols and organic solvents
Toxicity data	
LD ₅₀ oral	270 mg/kg rat

Antimicrobial effectiveness/applications

4-Fluoromethylphenylsulphonic acid amide is described as a broad spectrum microbicide, recommended mainly as a paint film fungicide especially in water based paints, where it achieves simultaneously in-can protection. This property of the microbicide points to the water solubility of the substance and accordingly to leachability out of paint films.

11. Carbamates

Carbamates comprise the esters and the salts of carbamic acids. This is a systematic classification from chemical aspects. Microbicides of this class of substances widely differ in terms of both efficacy and mechanisms of activity. The carbamic and dithiocarbamic acids, the basis of the carbamates, lack chemical stability; they occur only intermediately and disintegrate instantly to form carbon dioxide (CO₂), carbon disulphide (CS₂) and amine (see Figure 17).



$X = O \text{ or } S; A^+ = \text{metal kation}$

Figure 17 Hydrolysis/degradation of carbamates.

The present section also deals with such microbicides as contain the car-bamate grouping $\left(\begin{array}{c} \text{-N-C-X-} \\ | \quad || \\ \text{X} \end{array} \right)$; X = O or S within the molecule as a structural element, e.g. dithiocarbamic acid disulphides such as thiram. Other compounds of that nature, with a hydroxymethyl group on the N atom and thus able to release formaldehyde, are listed under 3. 'Formaldehyde releasing compounds':

- 3.4.11. = Sodium *N*-hydroxymethyl-*N*-methyldithiocarbamate
- 3.4.12. = *N*-hydroxymethyl-benzothiazoline-2-thione
- 3.4.13. = *N*-hydroxymethyl-5,6-dichlorobenzoxazolinone
- 3.4.14. = *N*-hydroxymethyl-5-chlorobenzoxazoline-2-thione.

Dazomet contains the dithiocarbamate grouping as well; as a reaction product of amine and formaldehyde it is however described under 3.3.25. = 3,5- dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione. Before dithiocarbamates gained importance as microbicides for the protection of materials they were known as potent, non-systemic fungicides for plant and crop protection. They are characterized as fungitoxic metal binding agents. A survey on the chemistry and mode of action of dithiocarbamate fungicides is given by Ludwig & Thorn (1960).

Microbicide group (substance class)	11. CARBAMATES
Chemical name	11.1. 3-Iodopropynylbutylcarbamate (IPBC)
Chemical formula	$\text{C}_8\text{H}_{12}\text{INO}_2$
Structural formula	$\text{C}_4\text{H}_9\text{-NH-CO-O-CH}_2\text{-C}\equiv\text{C-I}$
Molecular mass	281.07
CAS-No.	55406-53-6
EC-No.	259-627-5; No 56 in EEC Cosmetics Directive
EPA	approval for antimicrobial applications
TSCA	Listed on Chemical Inventory
Synonym/common name	3-iodopropargyl- <i>N</i> -butylcarbamate, 3-iodoprop-2-ynyl butylcarbamate
Supplier	ARCH, DEGUSSA-HÜLS, TROY
Chemical and physical properties	
Appearance	crystalline, off-white powder with a weak odour
Content (%)	min 97
Melting point °C	65–66
Bulk density g/l (20°C)	450
Vapour pressure hPa (20°C)	1.04×10^{-5}
Ignition temperature °C	385
Log POW	2.4
Stability	stable under normal conditions; decomposition at 180°C; hydrolyses in strong alkaline media
Solubility g/l (20°C)	0.168 in H_2O , ~720 in acetone, ~340 in ethanol, ~100 in propylene glycol, ~400 in dipropylene glycol, ~450 in ethylene glycol monobutyl ether, ~120 in solvesso 100, ~15 in white spirit
Toxicity data (source: ARCH)	
LD ₅₀ oral	1400 mg/kg rat
dermal	> 2000 mg/kg rabbit
LC ₅₀ on inhalation	689 mg/m ³ (4 h) for rats
Severe eye irritant; skin irritant; not a sensitiser.	
Not known to be a reproductive or developmental toxin. Not known to be carcinogenic or mutagenic.	
Ecotoxicity (source: BAYER):	
The product is classified as not easily biodegradable (result of the closed bottle test; duration: 28 days).	
Fish toxicity: LC ₀ for <i>Brachydanio rerio</i>	0.26 mg/l (96 h)
LC ₅₀	0.43 mg/l (96 h)
EC ₀ for <i>Daphnia magna</i>	0.11 mg/l (48 h)

EC ₅₀	0.21 mg/l (48 h)
EC ₀ for green algae (<i>Scenedesmus subs.</i>)	0.01 mg/l (72 h)
EC ₅₀	0.026 mg/l (72 h)
Waste water bacteria are inhibited (50% inhibition) by 44 mg a.i./l.	

Antimicrobial effectiveness/applications

IPBC is highly effective against a wide variety of fungal species, e.g. blue stain fungi (*Aureobasidium pullulans*, *Sclerophoma pityophila*), sapstainers (*Diplodia natalensis*, *Ceratocystis virescens*, *Ceratocystis pluriannulata*), wood rotting fungi, (*Coniophora puteana*, *Polyporus versicolor*, *Poria monticola*, *Gloeophyllum trabeum*, *Lenzites trabea*).

Table 91 Minimum inhibition concentrations (MIC) of IPBC in nutrient agar (Source: DEGUSSA-HÜLS)

Test organismen	MIC (mg/l)
Mold/Mildew/Yeast/Fungi	
<i>Alternaria tenuis</i>	5.0
<i>Aspergillus glaucus</i>	4.0
<i>Aspergillus niger</i>	0.6–5.0
<i>Aspergillus oryzae</i>	4.0
<i>Aureobasidium pullulans</i>	4.0–6.0
<i>Candida albicans</i>	6.0–8.0
<i>Chaetomium globosum</i>	5.0
<i>Gliocladium sp.</i>	8.0
<i>Penicillium brevicaulis</i>	1.0
<i>Penicillium funiculosum</i>	4.0–6.0
<i>Saccharomyces cerevisiae</i>	5.0
<i>Talaromyces flavus</i>	6.0
<i>Trichoderma viride</i>	10.0
Algae	
<i>Chlorella pyrenoidosa</i>	8.0
<i>Oscillatoria sp.</i>	< 50.0
Bacteria	
<i>Bacillus subtilis</i>	50.0
<i>Escherichia coli</i>	100.0
<i>Klebsiella pneumoniae</i>	50.0
<i>Pseudomonas aeruginosa</i>	250.0–1000.0

IPBC is used as a paint film fungicide and algicide in both solvent or water based surface coatings. It serves as an active ingredient in non-film forming decorative wood stains and can be formulated in wood preservative formulations suitable for the protection of freshly cut and sawn timber against staining fungi and moulds and for combating wood rotting fungi. Incorporating IPBC into solvent based systems, e.g. anti-blue-stain formulations, one has to pay attention to the fact that the agent may be incompatible with the driers. Other application fields for the fungicide are adhesives (wet products and films), plastics (at processing temperatures not higher than 177°C), textile and paper coatings, inks, metal working fluids.

IPBC's spectrum of effectiveness comprises also yeasts, e.g. *Candida albicans*, *Saccharomyces cerevisiae*, and in considerably higher concentrations bacteria, too. Therefore its use as a preservative for cosmetic and personal care products has become an additional application field. To achieve cost-efficient and effective preservation it is worth combining the microbicide with established cosmetic preservatives (see Part I—Chapter 5.9.). IPBC is listed in the EEC Cosmetics Directive; maximum permissible concentration: 0.05%. Excluded is the use of the product as an agent for oral hygiene or lip care products. If in leave-on cosmetics (retention on the skin) the IPBC concentration exceeds 0.02%, the note 'contains iodine' must be added.

Microbicide group (substance class)

Chemical name

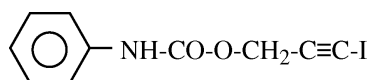
Chemical formula

Structural formula

11. CARBAMATES

11.2. 3-Iodopropynylphenylcarbamate (IPPC)

C₁₀H₈INO₂



Molecular mass	301.09
CAS-No.	22618-38-8
EC-No.	unknown
Synonym/common name	3-iodopropargyl-N-phenylcarbamate,3-iodoprop-2-ynyl phenylcarbamate
Chemical and physical properties	
Appearance	white crystalline powder
Content (%)	~100
Melting point °C	144–146
Stability	stable in solvent and water based formulations; hydrolyses in strong alkaline media
Solubility	virtually insoluble in water, highly soluble in aromatic and polar organic solvents; low solubility in aliphatic hydrocarbon solvents
Toxicity data	Not available.

Antimicrobial effectiveness/applications

As can be seen from Table 92, the antimicrobial activity of IPPC is similar to that of IPBC (11.1.). Accordingly IPPC could be used in fields of application which are described for IPBC. In exposure tests with paint films IPPC containing coatings appeared of somewhat longer lasting effectiveness in comparison to IPBC, which apparently is a little more leachable. However, up to now IPPC is not a commercially available microbicide.

Table 92 Minimum inhibition concentrations (MIC) of IPPC in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	3.5
<i>Aureobasidium pullulans</i>	5
<i>Aspergillus niger</i>	1
<i>Chaetomium globosum</i>	1
<i>Coniophora puteana</i>	0.5
<i>Lentinus tigrinus</i>	0.75
<i>Penicillium glaucum</i>	1.5
<i>Polyporus versicolor</i>	1
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	35
<i>Candida albicans</i>	10
<i>Rhodotorula rubra</i>	10
<i>Sporobolomyces roseus</i>	5
<i>Torula utilis</i>	3.5
<i>Escherichia coli</i>	200
<i>Staphylococcus aureus</i>	1000

Microbicide group (substance class)	11. CARBAMATES
Chemical name	11.3. 3-Iodopropynylcarbamate (IPC)
Chemical formula	$C_4H_4INO_2$
Structural formula	$H_2N-CO-O-CH_2-C\equiv C-I$
Molecular mass	224.99
CAS-No.	129348-50-1
EC-No.	unknown
Synonym/common name	3-iodopropargylcarbamate
Chemical and physical properties	
Appearance	white solid
Melting point °C	85–87

Stability	hydrolytically stable between pH 3 and 11
Solubility	sparingly soluble in water; soluble in organic solvents
Toxicity data	Not available

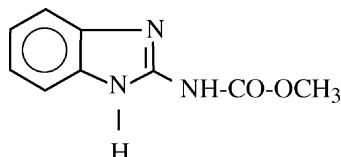
Antimicrobial effectiveness/applications

IPC is reported (Rayudu, 1988) to have a number of advantages over IPBC (11.1.) and IPPC (11.2.). It is an excellent microbicide and its antimicrobial activity covers both fungi and bacteria including slime forming micro-organisms. These properties open a variety of application fields to the microbicide IPC which are closed for instance to IPBC and IPPC. The compound may be used as a preservative for aqueous functional fluids, e.g. polymer emulsions, latex paints and metal working fluids. A further recommended use for IPC is the addition to pulp slurry or cooling water to inhibit the formation of slime. Additionally IPC protects coatings from mould growth after application. However, in accordance with its chemical structure it should be more leachable than IPBC and IPPC and therefore be able to act as an in-can preservative for aqueous functional fluids, which has been confirmed.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

11. CARBAMATES

11.4. Methyl-N-(2-benzimidazolyl)carbamate
 $C_9H_9N_3O_2$



Molecular mass	191.19
CAS-No.	10605-21-7
EC-No.	234-232-0
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	2-methoxycarbonylamino-benzimidazole, N-benzimidazol-2-ylcarbamic acid methylester (BCM), Carbendazim
Supplier	DUPONT, SCHIRM
Chemical and physical properties	
Appearance	white to off-white, odourless, crystalline powder
Content (%)	min. 99
Melting point °C	> 295 (decomposition)
Density g/ml (20°C)	approx. 1.5
Vapour pressure hPa (20°C)	< 2.6×10^{-6}
Bulk density g/l	approx. 500
Log POW at pH 7	1.6
pH in H ₂ O at 20°C	6.5
Stability	stable between pH 2 to 12; at pH > 13 slow hydrolysis to 2-aminobenzimidazole; thermal decomposition begins at 350°C; exothermic reaction with air supply (spontaneous combustion) starts at 400°C
Solubility g/l (24°C)	0.008 in H ₂ O; sparingly soluble in organic solvents; water soluble salts are formed in strong acids
Toxicity data (source: BAYER)	
LD ₅₀ oral	640 mg/kg rat 11000 mg/kg mouse
LD ₅₀ dermal	> 2000 mg/kg rat
No sensitization was observed in the guinea pig test. Not irritant to the skin; causes slight irritation of the eyes.	
Mutagenicity: mutagenic substance; proposal category 2.	

Ecotoxicity:

Result of the closed bottle test/BOD-determination:

classification 'not readily degradable'. Microbial degradation in soil: half-live 5–6 weeks.

LC₅₀ for fish (*Cyprinus carpio*) 0.61 mg/l (96 h)

EC₅₀ for *Daphnia magna* 0.22 mg/l (48 h)

EC₅₀ for green algae (*Scenedesmus subs.*) 419 mg/l (72 h)

Antimicrobial effectiveness/applications

The, antimicrobial activity of BCM and Benomyl (11.5.) is based on a severe inhibition of DNA synthesis, which takes place before other systems are affected (Clemons & Sisler, 1971).

BCM exhibits a striking activity against a variety of fungal species and yeasts, but is more or less ineffective against bacteria and algae. However, there are deep gaps in BCM's spectrum of efficacy (see Table 93). Another handicap of BCM is the poor solubility of the fungicide in solvents, making it difficult to formulate the active ingredient and additionally inhibiting its penetration, for example, into wood. The activity of BCM against wood staining fungi is excellent; it is therefore especially suitable for use in the formulation of anti-blue stain agents. In contrast to many other fungicides for the protection of materials BCM is also highly effective against *Trichoderma* species. The main faults are against species of *Alternaria*, *Mucor*, *Geotrichum*, *Streptovercillium*, *Cephaloscyus* and *Candida*. In cases where complete protection is required, e.g. in fungicidal coatings, impregnating agents, sealants and plastics, it makes sense to combine BCM with another fungicide which has the relevant spectrum of activity. Examples of fungicides which are able to fill in the gaps in the activity spectrum of BCM are *N*-trihalomethylthio compounds (16.), certain dithiocarbamates (11.), methylsulphonyl-tetrachloropyrimidine (13.3.), azole fungicides (14.) and others. In some cases of such combinations not only additivity of effectiveness is observed but synergism too (Brake, 1974; Paulus & Genth, 1986).

The applications of BCM as a microbicide for the protection of materials are determined by the fact that its useful properties – it is non-volatile, heat resistant, non-leaching and colour-fast – are counter-effected by its bad properties – it has an unequalized spectrum of activity and poor solubility.

Table 93 Minimum inhibition concentrations (MIC) of BCM in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	> 1000
<i>Aureobasidium pullulans</i>	0.1–0.5
<i>Aspergillus niger</i>	5–10
<i>Cephaloscyus fragrans hanava</i>	> 1000
<i>Ceratocystis pilifera</i>	0.5
<i>Chaetomium globosum</i>	0.5
<i>Cladosporium cladosporioides</i>	0.5
<i>Gliocladium virens</i>	1.0
<i>Lentinus tigrinus</i>	> 1000
<i>Penicillium glaucum</i>	0.5
<i>Phialophora fastigiata</i>	0.5
<i>Sclerophoma pityophila</i>	0.5
<i>Streptovercillium reticulum</i>	> 1000
<i>Trichoderma viride</i>	1–2
<i>Candida albicans</i>	> 1000
<i>Candida krusei</i>	> 1000
<i>Rhodotorula mucilaginosa</i>	7.5
<i>Rhodotorula rubra</i>	5
<i>Sporobolomyces roseus</i>	1–2

Microbicide group (substance class)

Chemical name

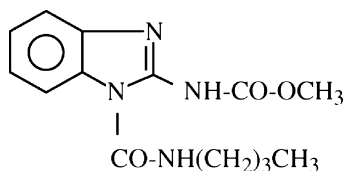
Chemical formula

11. CARBAMATES

11.5. Methyl-N-(1-butylcarbamoyl-) benzimidazol-2-ylcarbamate

C₁₄H₁₈N₄O₃

Structural formula



Molecular mass

290.33

CAS-No.

17804-35-2

EC-No.

241-775-7

EPA-Reg.

registered

Synonym/common name

Benomyl

Supplier

DUPONT

Chemical and physical properties

Appearance

colourless crystals

Content (%)

~100

Melting point °C

decomposes without melting

Stability

unstable in aqueous media, liberates Carbendazim (11.4.); at pH ≥ 13 quantitative conversion to 3-butyl-2,4-dioxo-s-triazino[1,2- α] benzimidazole (11.6 = STB) (Chiba & Singh, 1986); decomposition when heated (release of methanol and formation of STB)

Solubility g/l (25°C)

0.004 in H₂O, 18 in acetone, 400 in heptane

Toxicity data

LD₅₀ oral

5600 mg/kg mouse

> 10000 mg/kg rat

The toxicity of Carbendazim which is set free in aqueous media has to be taken into consideration. Benomyl is mildly irritant to skin and mucosa.

Antimicrobial effectiveness/applications

As Benomyl in aqueous media always splits into BCM and *N*-butylcarbamic acid which is very reactive and unstable (decomposition to butylamin and CO₂), the antimicrobial efficacy of the fungicide corresponds to BCM (11.4.). Even in solutions of Benomyl in organic solvents there exists an equilibrium between Benomyl on one side and BCM and butyl isocyanate on the other side; precipitation of BCM due to poor solubility in organic solvents shifts the equilibrium further to the side of BCM. Also STB (11.6.) which is formed from Benomyl at pH 13 or on heating exhibits an antimicrobial activity spectrum similar to that of BCM. As Benomyl apparently is a BCM (or STB) releasing compound having no remarkable advantages over BCM, it has not found much interest as a microbicide for the protection of materials. Benomyl has gained importance for plant protection as a fungicide which is systemic in plant tissue.

Microbicide group (substance class)

11. CARBAMATES

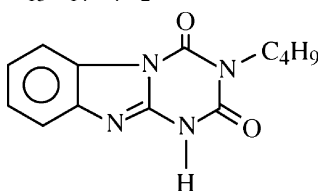
Chemical name

11.6. 3-Butyl-2,4-dioxo-s-triazino[1,2- α]benzimidazole (STB)

Chemical formula

C₁₃H₁₄N₄O₂

Structural formula



Molecular mass

258.28

CAS-No.

41136-38-3

EC-No.

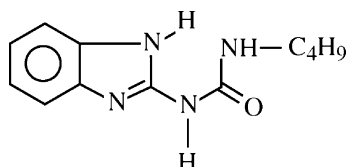
unknown

Chemical and physical properties

Appearance

crystalline powder

Content (%)	~100
Melting point °C	280
Stability	The triazine derivative is mentioned under carbamates, as it originates from Benomyl (11.5.) and at $\text{pH} \geq 13$ intermediarily hydrolyzes to an unstable carbamic acid which after elimination of CO_2 stabilizes to 1-(2-benzimidazolyl)-3-n-butylurea (Chiba & Singh, 1986); structural formula:



Solubility	virtually insoluble in H_2O , moderately soluble in acetonitrile, DMF, DMSO; in alkalis conversion to water soluble carbamic acid alkali salts

Toxicity data – Not available.

Antimicrobial effectiveness (see Table 94)

Table 94 Minimum inhibition concentrations (MIC) of STB in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	10–20
<i>Chaetomium globosum</i>	0.5
<i>Penicillium glaucum</i>	5
<i>Rhizopus nigricans</i>	> 2000

Microbicide group (substance class)

Chemical name

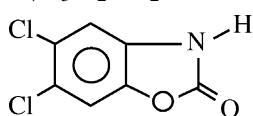
Chemical formula

Structural formula

11. CARBAMATES

11.7. 5,6-Dichlorobenzoxazolinone

$\text{C}_7\text{H}_3\text{Cl}_2\text{NO}_2$



Molecular mass

CAS-No.

EC-No.

204.01

5285-41-6

unknown

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Bulk density g/l

Stability

greyish powder with a characteristic odour

~100

205–206

240

conversion to soluble salts in alkaline media; on boiling in alkaline solutions hydrolysis to 3, 4-dichloro-6-aminophenol

Solubility g/l (25°C)

0.25 in H_2O , ~60 in ethanol, isopropanol, propylene glycol, 120 in methyl ethyl ketone, 4.8 in toluene

Toxicity data

LD_{50} oral

1100 mg/kg mouse

Not irritant to the skin.

Antimicrobial effectiveness/application

5, 6-Dichlorobenzoxazolinone is a very potent fungicide which had been used in the past mainly for the protection of textile material against biodeterioration. The minimum inhibition concentrations of the compound for a wide variety of fungal species are in the range of 10–100 mg a.i./ml nutrient agar. However, 5,6-dichlorobenzoxazolinone is no longer of practical importance.

Microbicide group (substance class)

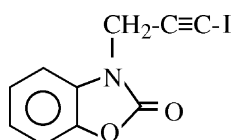
Chemical name

Chemical formula

Structural formula

11. CARBAMATES

11.8. 3-(3-Iodopropargyl) benzoxazol-2-one

 $C_{10}H_6INO_2$ 

Molecular mass

299.07

CAS-No.

135306-48-8

EC-No.

unknown

Synonym/common name

3-(3-iodo-2-propynyl)benzoxazol-2-one

Melting point °C

162–164

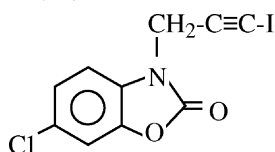
Chemical name

11.9. 3-(3-Iodopropargyl)-6-chlorobenzoxazol-2-one

Chemical formula

 $C_{10}H_5ClINO_2$

Structural formula



Molecular mass

333.52

CAS-No.

135306-49-9

EC-No.

unknown

Synonym/common name

3-(3-Iodo-2-propynyl)-6-chloro-benzoxazol-2-one

Antimicrobial effectiveness/application

The microbicides mentioned under 11.8. and 11.9. are novel benzoxazolone compounds the antimicrobial activity of which is demonstrated by the MIC's listed in Table 95. The microbicides are highly effective against moulds and yeasts. Their utility as microbicides is described by Chi-Tung Hsu (1991).

Table 95 Minimum inhibition concentrations (MIC) of 11.8. and 11.9.

Test organism	MIC (mg/litre)	
	11.8.	11.9.
<i>Aspergillus niger</i>	< 0.13	< 0.13
<i>Aureobasidium pullulans</i>	0.25	≤ 1
<i>Cladosporium resinae</i>	≤ 5	≤ 1
<i>Gloeophyllum trabeum</i>	≤ 0.2	≤ 1
<i>Penicillium funiculosum</i>	≤ 1	≤ 1
<i>Rhodotorula rubra</i>	≤ 1	≤ 1
<i>Saccharomyces cerevisiae</i>	≤ 1	≤ 5
<i>Escherichia coli</i>	> 250	> 250
<i>Pseudomonas aeruginosa</i>	> 250	> 250
<i>Pseudomonas fluorescens</i>	> 250	16
<i>Staphylococcus aureus</i>	> 250	16

Microbicide group (substance class)	11.10. SALTS OF N-METHYLDITHIOCARBAMIC ACID
Chemical name	11.10.1. Sodium-N-methyldithiocarbamate
Chemical formula	$C_2H_4NNaS_2$
Structural formula	$ \begin{array}{c} H_3C-NH-C-S^-Na^+ \\ \\ S \end{array} $
Molecular mass	129.18
CAS-No.	137-42-8
EC-No.	205-293-0
Synonym/common name	methyl-carbamodithioic acid sodium salt, Metam
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	~100
Stability	decomposes when heated and in acid media; can cause colouration on contact with heavy metals
Solubility	highly soluble in water and mixtures of water and alcohols
Toxicity data	
LD ₅₀ oral	300–800 mg/kg rat
Strongly irritant to the skin and eyes.	

Antimicrobial effectiveness/applications

The antimicrobial activity corresponds to that of the potassium salt (see 11.10.2.). For practical application – controlling bacterial and fungal slime – in general a 40% aqueous solution of the sodium salt is available.

Microbicide group (substance class)	11.10. SALTS OF N-METHYLDITHIOCARBAMIC ACID
Chemical name	11.10.2. Potassium N-methyldithiocarbamate
Chemical formula	$C_2H_4KNS_2$
Structural formula	$ \begin{array}{c} H_3C-NH-C-S^-K^+ \\ \\ S \end{array} $
Molecular mass	145.28
CAS-No.	137-41-7
EC-No.	205-292-5
EPA	approval for antimicrobial application
Synonym/common name	methyl-carbamodithioic acid potassium salt
Supplier	BUCKMAN
Chemical and physical properties of a 40% aqueous solution	
Appearance	clear, coloured (orange to red or yellow to green) fluid with a slight sulphurous odour
Boiling point/range °C (101 kPa)	> 100
Solidification point °C	< –15
Density g/ml (25°C)	1.23
Viscosity mPas (20°C)	< 10
Flash point °C	closed cup: > 100
pH (100 mg/l H ₂ O)	8–10
Stability	highly reactive (decomposition) with acids; on contact with heavy metals coloured reaction products may be formed; decomposition temperature ~70°C
Solubility	easily soluble in cold and hot water and in mixtures of water and alcohols

Toxicity data (source: BUCKMAN)

LD ₅₀ oral	630 mg/kg rat
dermal	> 2000 mg/kg rabbit
Corrosive to skin and eyes.	
Classified as a sensitizer.	

Antimicrobial effectiveness/application

Potassium N-methyldithiocarbamate is a broad spectrum microbicide. As it is of limited stability, it is mainly used for short-term control of microbial contamination.

Microbicide group (substance class)	11.11. SALTS OF N,N-DIMETHYLDITHIOCARBAMIC ACID
Chemical name	11.11.1. Sodium dimethyldithiocarbamate
Chemical formula	C ₃ H ₆ NNaS ₂
Structural formula	$ \begin{array}{c} (\text{H}_3\text{C})_2\text{N}-\text{C}-\text{S}^-\text{Na}^+ \\ \parallel \\ \text{S} \end{array} $
Molecular mass	143.21
CAS-No.	128-04-1
EC-No.	204-876-7
USA EPA TSCATS	Data Base Jan. 2001
FDA	listed as a component of process aids in sugar manufacture, of slimicides in the manufacture of paper and cardboard in indirect contact with foodstuffs, of adhesives based on polymers in indirect contact with foodstuffs
JAPAN MITI	No.: 2-1249 and 2-1833
Synonym/common name	dimethyldithiocarbamic acid sodium salt, Dibam
Supplier	BAYER, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	light-yellow liquid with a weak sulphurous odour
Content (%)	40–42
Crystallisation point/range °C	approx. 5
Melting point °C	approx. –5
Density g/ml (20°C)	approx. 1.17–1.185
Vapour pressure hPa (20°C)	20
Viscosity mPa·s (20°C)	4.8
Ignition temperature °C	not below 500
pH (1% in H ₂ O)	approx. 9–12
Stability	stable at pH 7–13; not stable in acid solutions; thermal decomposition at temperatures above 100°C: carbon disulphide, hydrogen sulphide and dimethylamine can occur via an endothermic reaction; in contact with Fe, Cu, Sn, Pb and Co deeply coloured, insoluble salts may come into being
Solubility	readily miscible with water and lower alcohols
Toxicity data (source: BAYER)	
LD ₅₀ oral	3591 mg/kg rat
dermal	> 5000 mg/kg rat (24 h)
Toxicity of the decomposition product CS ₂ :	
LC ₅₀ on inhalation for rats	25 mg/l = 7900 ppm (2 h)
for mice	10 mg/l = 3160 ppm (2 h)
Rabbit skin exposed to the product for 4 hours showed no signs of irritation; however, the product turned out to be slightly irritant to rabbit eyes.	

Ecotoxicity:

The activity of activated sludge organisms in waste water treatment plants is not impaired at concentrations of 50 mg/l.

EC ₅₀ for algae (<i>Chlorella pyrenoidosa</i>)	0.8 mg a.i./l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	0.67 a.i./l (48 h)
LC ₀ for <i>Leuciscus idus</i>	~1 mg a.i./l (48 h)
LC ₅₀ for <i>Leuciscus idus</i>	~2.2 mg a.i./l (48 h)
LC ₅₀ for <i>Poecilia reticulata</i>	~2.6 mg a.i./l (96 h)

Antimicrobial effectiveness/application

In the sugar industry the proliferation of thermophilic bacteria, mainly *Leuconostoc* and *Bacillus* strains, may cause sugar loss during Saccharose extraction from sugar beet or sugar cane. Due to its broad spectrum of effectiveness (see Table 96) the sodium dimethyldithiocarbamate solution is capable to prevent the sugar degradation in the raw iuce at low addition rates (15–25 ppm), thereby improving the cost effectiveness of the sugar extraction process. The active ingredient decomposes relatively quickly under the process conditions, especially with increasing temperature, and accordingly is not retained by the sugar.

Table 96 Minimum inhibition concentrations (MIC) of a 42% solution of sodium *N*-dimethyldithiocarbamate in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria tenuis</i>	200
<i>Aspergillus niger</i>	> 1000
<i>Aureobasidium pullulans</i>	500
<i>Chaetomium globosum</i>	500
<i>Cladosporium cladosporioides</i>	500
<i>Lentinus tigrinus</i>	200
<i>Penicillium glaucum</i>	500
<i>Sclerophoma pityophila</i>	150
<i>Trichoderma viride</i>	> 1000
<i>Candida albicans</i>	500
<i>Candida krusei</i>	500
<i>Rhodotorula mucilaginosa</i>	500
<i>Saccharomyces bailii</i>	200
<i>Saccharomyces cerevisiae</i>	500
<i>Torula rubra</i>	500
<i>Torula utilis</i>	500
<i>Aerobacter aerogenes</i>	150
<i>Aeromonas punctata</i>	200
<i>Bacillus mycoides</i>	100
<i>Bacillus subtilis</i>	200
<i>Escherichia coli</i>	500
<i>Leuconostoc mesenteroides</i>	500
<i>Proteus mirabilis</i>	> 1000
<i>Pseudomonas aeruginosa</i>	> 1000
<i>Pseudomonas fluorescens</i>	> 1000
<i>Staphylococcus aureus</i>	500

Another application field for sodium dimethyldithiocarbamate is the leather industry where it may be used to prevent the growth of bacteria in the soaking liquor and microbial damage to hides. Addition rates move between 0.05–0.1% of the solution containing 40–42% active ingredient.

The broad spectrum microbicide may also be applicated for controlling the growth of bacteria and fungi in pulp and paper manufacture and related systems. It is employed not only as a slimicide but also for the protections of formulations used for paper making, e.g. glues, starch and clay slurries, and coating preparations. But when adding sodium dimethyldithiocarbamate to such formulations one has to bear in mind, that traces of heavy metals can cause colorations. For slime control one usually adds 50–200 mg/l water, in pulp and paper mills 20–80 kg/100 t of dry pulp or paper per day.

Microbicide group (substance class)	11.11. SALTS OF N,N-DIMETHYLDITHIO-CARBAMIC ACID
Chemical name	11.11.2. Potassium dimethyldithiocarbamate
Chemical formula	C ₃ H ₆ KNS ₂

Structural formula	$\begin{array}{c} (\text{H}_3\text{C})_2\text{N}-\text{C}-\text{S}^-\text{K}^+ \\ \parallel \\ \text{S} \end{array}$
Molecular mass	159.32
CAS-No.	128-03-0
EC-No.	204-875-1
EPA	approval for antimicrobial application
Synonym/common name	dimethyl-carbamodithioc acid sodium salt
Supplier	BUCKMAN
Chemical and physical properties	
Appearance	light yellow to green aqueous fluid with a weak sulphurous odour
Content (%)	50
Boiling point/range °C (101 kPa)	> 100
Solidification point °C	-15
Viscosity mPa·s (20°C)	< 10
Flash point °C	closed cup: > 100
pH	> 12
Stability	in acid media intermediately the unstable dimethyldithiocarbamic acid is formed which disintegrates to CS ₂ , H ₂ S and dimethylamine; thermal decomposition starts at 70°C; colouration may occur in contact with heavy metals
Solubility	easily soluble in H ₂ O and aqueous alcohols
Toxicity data (source: BUCKMAN)	
LD ₅₀ oral	1867 to 2196 mg/kg rat
LD ₅₀ dermal	> 2990 to 3162 mg/kg rabbit
Irritating to skin; corrosive to eyes; may cause sensitization in case of skin contact.	
Ecotoxicity:	
LC ₅₀ for Trouts	0.4 mg/l (96 h)
LC ₅₀ for Daphnia magna	0.3 mg/l (48 h)
Biodegradable (Bunch-Chambers).	

Antimicrobial effectiveness/application

Potassium dimethyldithiocarbamate is broad spectrum microbicide as is the corresponding sodium salt. The 50% solution of the active ingredient is mainly applied in the leather industry and the pulp and paper industry (see under 11.11.1.).

Microbicide group (substance class)	11.11. SALTS OF N,N-DIMETHYLDITHIO-CARBAMIC ACID
Chemical name	11.11.3. Zinc dimethyldithiocarbamate
Chemical formula	C ₆ H ₁₂ N ₂ S ₄ Zn
Structural formula	$\left[\begin{array}{c} (\text{CH}_3)_2\text{N}-\text{C}-\text{S}^- \\ \parallel \\ \text{S} \end{array} \right]_2 \text{Zn}^{2+}$
Molecular mass	305.83
CAS-No.	137-30-4
EC-No.	205-288-3
EPA TSCA TS	Data base Jan. 2001
Synonym/common name	dimethylcarbamodithioic acid zinc salt, zinc bis(dimethyldithiocarbamate), Ziram
Supplier	DUPONT, SIGMA-ALDRICH

Chemical and physical properties

Appearance	white to grey odourless powder
Content (%)	> 90%
Melting point °C	250–252
Density g/ml (25°C)	1.66
Ignition temperature °C	> 300
Stability	stable between pH 5 and 10; decomposition in acid media with release of CS ₂ and dimethylamine; causes colouration when coming in contact with traces of heavy metals, e.g. Cu, Fe; disturbs/inhibits the oxidative drying of resins (alkyds)
Solubility g/l (25°C)	0.065 in H ₂ O; sparingly soluble in organic solvents

Toxicity data

LD ₅₀ oral	267 mg/kg rat
dermal	> 6000 mg/kg rat
intraperitoneal	8.7 mg/kg rat
subcutaneous	69 mg/kg rat
LC ₅₀ on inhalation	26 mg/m ³ (2 h)
LD ₅₀ intravenous	18 mg/kg mouse

Ziram has not yet been classified by EPA with regard to its carcinogenicity. Lab. trials showed mutagenic activity. The microbicide does not cause skin irritation, but irritates mucous membranes and eyes. No sensitization was observed in the guinea pig test.

Antimicrobial effectiveness/application

At first Ziram was developed for the application as a leaf fungicide for plant protection. However, it displays an unusually broad spectrum of activity covering fungi, yeasts, bacteriae and algae (see Table 97); additionally it is non-volatile, non-leaching and relatively heat resistant. Due to these properties it can be regarded as a microbicide suitable for use in coatings, plaster, adhesives, rubber and sealing compounds, cement jointing filler, particle and fiber board. The addition rates range between 0.2 and 1%.

Table 97 Minimum inhibition concentrations (MIC) of Ziram in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	200
<i>Aspergillus flavus</i>	200
<i>Aspergillus niger</i>	200
<i>Aspergillus terreus</i>	750
<i>Aureobasidium pullulans</i>	100
<i>Chaetomium globosum</i>	75
<i>Cladosporium herbarum</i>	100
<i>Coniophora puteana</i>	< 20
<i>Lentinus tigrinus</i>	200
<i>Paecilomyces variotii</i>	200
<i>Penicillium citrinum</i>	500
<i>Penicillium glaucum</i>	150
<i>Polyporus versicolor</i>	200
<i>Rhizopus nigricans</i>	75
<i>Trichoderma viride</i>	200
<i>Trichophyton pedis</i>	100
<i>Candida albicans</i>	350
<i>Candida krusei</i>	200
<i>Rhodotorula mucilaginosa</i>	150
<i>Rhodotorula rubra</i>	200
<i>Sporobolomyces roseus</i>	50
<i>Torula rubra</i>	200
<i>Torula utilis</i>	200
<i>Bacillus subtilis</i>	150
<i>Escherichia coli</i>	50
<i>Pseudomonas aeruginosa</i>	75
<i>Staphylococcus aureus</i>	200
Algae	1

Microbicide group (substance class)	11.12. SALTS OF ETHYLENE-1,2-BISDITHIOCARBAMIC ACID
Chemical name	11.12.1. Disodium ethylene-1,2-bisdithiocarbamate
Chemical formula	$C_4H_6N_2Na_2S_4$
Structural formula	$Na^+ \begin{array}{c} \text{S-C-NH-CH}_2\text{-CH}_2\text{-NH-C-S}^- \\ \qquad \qquad \qquad \\ S \qquad \qquad \qquad S \end{array} Na^+$
Molecular mass	256.35
CAS-No.	142-59-6
EC-No.	205-547-0
EPA TSCA TS	Data base, Jan. 2001
Synonym/common name	ethylenebis(dithiocarbamic acid) disodium salt, Nabam
Supplier	FLUKA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	solid
Content (%)	~90
Stability	decomposes on heating without melting
Solubility	soluble in H ₂ O and aqueous alcohols
Toxicity data	
LD ₅₀ oral	395 mg/kg rat 580 mg/kg mouse
LD ₅₀ intraperitoneal	500 mg/kg rat
Irritant to skin and mucous membranes.	

Antimicrobial effectiveness/application

Nabam was developed by ROHM & HAAS (1943) as a fungicide for the application in soil and for the protection of seed against moulding. It also has been applied in rice cultures, because of its strong efficacy against algae. However, as a microbicide for the protection of materials Nabam has not gained importance.

Microbicide group (substance class)	11.12. SALTS OF ETHYLENE-1,2-BIS-DITHIOCARBAMIC ACID
Chemical name	11.12.2 Zinc ethylenebisdithiocarbamate
Chemical formula	$C_4H_6N_2S_4Zn$
Structural formula	$\left[\begin{array}{c} H_2C-NH-CS-S^- \\ \\ H_2C-NH-CS-S^- \end{array} \right] Zn^{2+}$
Molecular mass	275.76
CAS-No.	12122-67-7
EC-No.	235-180-1
Synonym/common name	Zineb
Supplier	DUPONT, RIEDEL DE HAEN
Chemical and physical properties	
Appearance	yellowish powder with an unpleasant odour
Content (%)	~100
Melting point °C	240 (decomposition)
Vapour pressure hPa (20°C)	< 0.001
Stability	decomposition at 157°C without melting; unstable in acid media
Solubility	virtually insoluble in H ₂ O, only moderately soluble in organic solvents

Toxicity data

LD ₅₀ oral	1850 mg/kg rat 7600 mg/kg mouse 4450 mg/kg rabbit
LD ₅₀ intraperitoneal	1940 mg/kg mouse
LD ₅₀ cutaneous	> 2500 mg/kg rat

Irritant to skin and mucous membranes.

May cause sensitization.

Classified as carcinogenic by RTECS criteria.

Antimicrobial effectiveness/applications

Although Zineb displays a broad spectrum of effectiveness and considerable antimicrobial activity, it has not often been employed as a microbicide for the protection of materials. The stable Ziram (11.11.3.) is the preferred zinc dithiocarbamate for that application.

Table 98 Minimum inhibition concentrations (MIC) of Zineb in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria tenuis</i>	10
<i>Aspergillus niger</i>	50
<i>Aureobasidium pullulans</i>	5
<i>Chaetomium globosum</i>	200
<i>Coniophora putena</i>	2
<i>Lentinus tigrinus</i>	5
<i>Penicillium glaucum</i>	20
<i>Polyporus versicolor</i>	50
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	500
<i>Escherichia coli</i>	100
<i>Staphylococcus aureus</i>	100
Algae	20–100

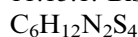
Microbicide group (substance class)

11.13. BIS(ALKYLTHIOCARBAMOYL)-DISULPHIDES

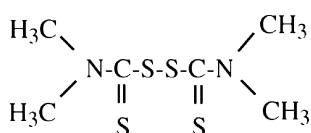
Chemical name

11.13.1. Bis(dimethylcarbamoyl)disulphide

Chemical formula



Structural formula



Molecular mass

240.43

CAS-No.

137-26-8

EC-No.

205-286-2

EPA TSCATS

Data base Jan. 2001

Synonym/common name

tetramethylthiuram disulphide, bisdimethylthiocarbamoyl disulfane, Thiram

Supplier

BAYER, DUPONT, FLUKA

Chemical and physical properties

Appearance

colourless, odourless crystals

Content (%)

> 98

Melting point °C

147–152

Density g/ml (20°C)	~1.77 (of a 50% aqueous dispersion)
Vapour pressure hPa (20°C)	$< 10^{-4}$
Viscosity mPas (23°C)	1500–3000 (of a thixotropic 50% aqueous) dispersion
pH of a 10% suspension in H ₂ O	approx. 9
Stability	stable between pH 4.5–9; decomposition in acid media with release of carbon disulphide (CS ₂) and dimethylamine; Thiram causes staining on contact with heavy metals, e.g. iron, copper, bronze or brass
Solubility g/l	0.03 in H ₂ O, 25 in acetone, 38 in ethanol, 75 in dimethylformamide; moderately soluble in benzene, highly soluble in CS ₂

Toxicity data

LD ₅₀ oral	560 mg/kg rat
intraperitoneal	138 mg/kg rat
subcutaneous	646 mg/kg rat
LC ₅₀ on inhalation	500 mg/m ³ (4 h) for rats
LD ₅₀ oral	1250 mg/kg mouse

In tests with rabbits non irritant to the skin, but serve irritation of the eyes (cauterizes the cornea). In susceptible people sensitization is possible. Thiram is classified as possibly mutagenic.

Exposure limits (occupational) France/Germany/UK 5 mg/m³

Ecotoxicity (source BAYER):

Result of the closed bottle test with adapted bacteria (BOD-determination): moderately degradable (above 45%).

LC ₅₀ for fish; <i>Brachydanio rerio</i>	0.035 mg/l (98 h)
<i>Leuciscus idus</i>	2 mg/l (48 h)

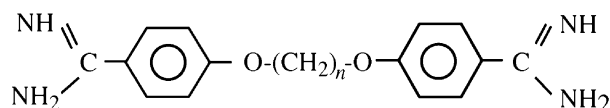
50% respiratory inhibition of activated sludge organisms is effected by 40 mg/l (test conforming to OECD Guidline 209).

Antimicrobial effectiveness/application

With regard to fungi and yeasts Thiram's spectrum of activity is considerably equalized. But deep gaps occur in the activity spectrum for bacteria; pseudomonads especially resist Thiram (see Table 99). The compound therefore is not appropriate for the in-can protection of functional fluids. However, Thiram is an important paint film fungicide and algicide, as it is virtually insoluble in water, light-stable and non-volatile. It can be used in emulsion paints and in solvent based coatings as long as they contain no binders which dry by oxidation (Thiram disturbs/inhibits the drying of such binders). Thiram containing coatings should not be used for outdoor applications as staining may be caused by splashes of water transporting traces of heavy metals, e.g. rainwater from gutters. Mould resistant coatings based on Thiram are mainly applied in locations with high humidity, e.g. in the food industry, in bathrooms etc.. Paper, cardboard, shoe insoles and glues are other examples of materials which may be protected by Thiram against biodeterioration.

12. Dibenzamidines

Dibenzamidines of the following general structure

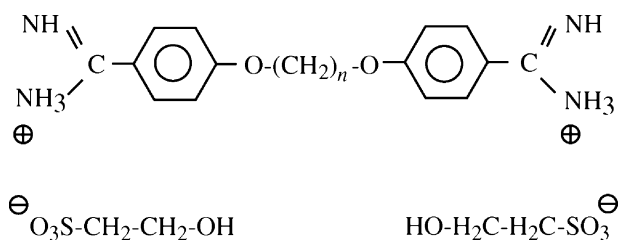


possess striking antimicrobial properties (Wien et al., 1948). They have a cationic character and in consequence belong to the membrane-active microbicides. Their main application as microbicides is in antiseptics; additionally they are used to some extent as preservatives for cosmetics. They have not gained importance as microbicides for the protection of materials, although their activity spectrum covers not only bacteria but fungi, too. Dibenzamidines in general are of poor water-solubility, but because of their aminic character they are able to

Table 99 Minimum inhibition concentrations (MIC) of Thiram in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	35
<i>Aspergillus niger</i>	150
<i>Aspergillus ustus</i>	50
<i>Aureobasidium pullulans</i>	25
<i>Cephaloscyus fragrans</i>	50
<i>Chaetomium globosum</i>	50
<i>Cladosporium cladosporioides</i>	25
<i>Cladosporium herbarum</i>	5
<i>Coniophora puteana</i>	1
<i>Fusarium culmorum</i>	75
<i>Fusarium moniliforme</i>	50
<i>Fusarium solani</i>	50
<i>Lentinus tigrinus</i>	25
<i>Paecilomyces variotii</i>	2.5
<i>Penicillium citrinum</i>	1000
<i>Penicillium glaucum</i>	50
<i>Phialophora fastigiata</i>	100
<i>Polyporus versicolor</i>	50
<i>Rhizopus nigricans</i>	25
<i>Rhizopus stolonifer</i>	100
<i>Sclerophoma pityophila</i>	10
<i>Stachybotris chartarum</i>	500
<i>Trichoderma viride</i>	175
<i>Trichophyton pedis</i>	250
<i>Candida albicans</i>	150
<i>Candida krusei</i>	100
<i>Rhodotorula mucilaginosa</i>	100
<i>Saccharomyces bailii</i>	50
<i>Saccharomyces cerevisiae</i>	50
<i>Sporobolomyces roseus</i>	50
<i>Torula rubra</i>	100
<i>Torula utilis</i>	100
<i>Aerobacter aerogenes</i>	50
<i>Aeromonas punctata</i>	75
<i>Bacillus mycoides</i>	25
<i>Bacillus subtilis</i>	100
<i>Desulfovibrio desulfuricans</i>	5
<i>Escherichia coli</i>	50
<i>Leuconostoc mesenteroides</i>	50
<i>Proteus mirabilis</i>	> 2500
<i>Proteus vulgaris</i>	> 2500
<i>Pseudomonas aeruginosa</i>	> 2500
<i>Pseudomonas fluorescens</i>	> 2500
<i>Staphylococcus aureus</i>	35

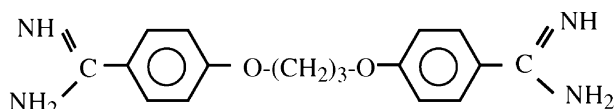
form water soluble salts with acids. The dibenzamidine salts of 2-hydroxyethanesulphonic acid, the isethionates, are of practical importance.



Microbicide group (substance class)
 Chemical name
 Chemical formula

12. DIBENZAMIDINES
 12.1. 4,4'-Diamidinophenoxypropane
 $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_2$

Structural formula



Molecular mass

312.38

CAS-No.

104-32-5

Synonym/common name

4,4'-Trimethylenedioxy)dibenzamidine, Propamidine

Chemical name

12.1a. 4,4'-Trimethylenedioxy)dibenzamidine bis(β -hydroxyethanesulphonate)

Chemical formula

 $C_{21}H_{32}N_4O_{10}S_2$

Molecular mass

564.65

Synonym/common name

Propamidine diisethionate

Chemical and physical properties

Appearance

white, crystalline, hygroscopic, odourless powder

Content (%)

~100

Melting point °C

235

pH (5% in H_2O)

4.5–6.5

Stability

precipitation and inactivation in the presence of phosphates; soy bean lecithin antagonizes the antifungal activity of Propamidine diisethionate

Solubility g/l

200 in H_2O ; highly soluble in alcohol

Toxicity data

LD₅₀ subcutaneous
intravenous

55 mg/kg mouse

42 mg/kg mouse

Antimicrobial effectiveness/application

The minimum inhibition concentrations vary from 5 mg/litre against *Staphylococcus aureus* to 75 mg/litre against *Pseudomonas aeruginosa* and to 500 mg/litre against *Aspergillus niger*. The activity is reduced by blood or serum and at low pH values.

Propamidine is a component of different antiseptics and sometimes a preservative in cosmetics (concentration ~0.1%). However, Propamidine is not listed in the EEC Cosmetics Directive.

Microbicide group (substance class)

12. DIBENZAMIDINES

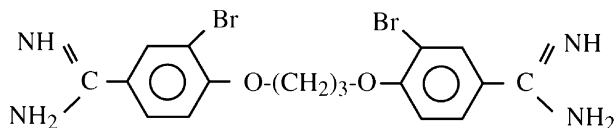
Chemical name

12.2. 4,4'-Diamidino-2,2'-dibromodiphenoxypropane

Chemical formula

 $C_{17}H_{18}Br_2N_4O_2$

Structural formula



Molecular mass

470.17

CAS-No.

496-00-4

Synonym/common name

4,4'-(trimethylenedioxy)-bis(3-bromobenzamidine),
Dibromopropamidine

Chemical name

12.2a. 4,4'-diamidino-2,2'-dibromodiphenoxypropane
bis(β -hydroxyethanesulphonate)

Chemical formula

 $C_{21}H_{30}Br_2N_4O_{10}S_2$

Molecular mass

722.47

CAS-No.

614-87-9

Synonym/common name

Dibromopropamidine diisethionate

Supplier

MAY and BAKER

Chemical and physical properties

Appearance

white, crystalline powder

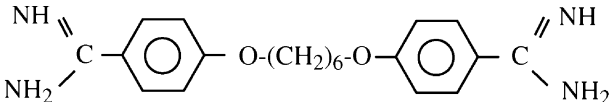
Content (%)

~100

Melting point °C	226
Stability	tolerates heating to 100°C for 30 min; minor decomposition during storage; incompatible with chloride, sulphate ions and anionic detergents
Solubility	highly soluble in H ₂ O and alcohols, soluble in glycerine, insoluble in oils
Toxicity data	
LD ₅₀ subcutaneous	300 mg/kg mouse
intravenous	10 mg/kg mouse
Irritant to skin and mucosa.	

Antimicrobial effectiveness/application

The bromination of Propamidine to Dibromopropamidine is accompanied by a considerable increase in antimicrobial activity. However the difference in efficacy against gram-positive and gram-negative bacteria remains unchanged; MIC for *Staphylococcus aureus* ~1 mg/l, MIC for *Proteus vulgaris* ~130 mg/l. Blood and serum reduce the antimicrobial effectiveness. Dibromopropamidine or the diisethionate of it is no longer listed in the EEC Cosmetics Directive.

Microbicide group (substance class)	12. DIBENZAMIDINES
Chemical name	12.3. 4,4'-(Hexamethylenedioxy)dibenzamidine
Chemical formula	C ₂₀ H ₂₆ N ₄ O ₂
Structural formula	
Molecular mass	354.46
CAS-No.	3811-75-4
EC-No.	no EC-no; EEC-no. 47
Synonym/common name	4,4'-Diamidinophenoxyhexane, Hexamidine
Supplier	AVENTIS, MAY and BAKER
Chemical name	12.3a. 4,4'-(Hexamethylenedioxy)dibenzamidinebis(β-hydroxyethanesulphonate)
Chemical formula	C ₂₄ H ₃₈ N ₄ O ₁₀ S ₂
Molecular mass	606.72
CAS-No.	659-40-5
Synonym/common name	4,4'-diamidinophenoxyhexane diisethionate, Hexamidine diisethionate
Chemical and physical properties	
Appearance	white, crystalline, odourless powder
Content (%)	~100
Stability	heat resistance < 100°C; incompatible with chloride and sulphate ions and anionic detergents
Solubility	fairly soluble in water and alcohol, insoluble in oils
Toxicity data	
LD ₅₀ oral	500–750 mg/kg mouse, rabbit or rat
Moderately irritant to skin and mucosa; sensitization and photosensitization effects were not observed.	

Antimicrobial effectiveness/application

The antibacterial activity of Hexamidine and its salts (in particular against Gram-positive bacteria) exceeds its effectiveness against moulds and yeasts. Optimum pH range 5–9. The activity is reduced in the presence of protein. In the EEC Cosmetic Directive the microbicide is mentioned under No. 47 as a preservative with a maximum permitted concentration of 0.1%.—Percentage of use in US cosmetic formulations: 0.02%.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical name

Chemical formula

Molecular mass

CAS-No.

Synonym/common name

Chemical and physical properties

Solubility

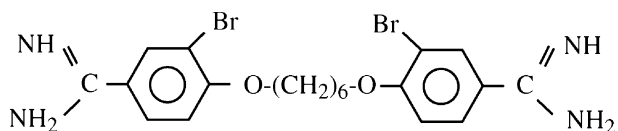
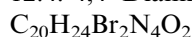
Toxicity data

LD₅₀ oral

cutaneous

intravenous

Irritant to skin and mucosa. No sensitization.

12. DIBENZAMIDINES**12.4. 4,4'-Diamidino-2,2'-dibromodiphenoxyhexane**

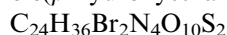
512.25

93856-82-7

No EC-no.; EEC-no. 15

4,4'-(hexamethylenedioxy)-bis(3-bromobenzamidine),
Dibromohexamidine

MAY and BAKER

**12.4a. 4,4'-Diamidino-2,2'-dibromodiphenoxyhexane
bis(β-hydroxyethanesulphonate)**

764.55

93856-83-8

Dibromohexamidine diisethionate

soluble in H₂O, alcohol, glycerine, virtually insoluble in
benzene

> 4000 mg/kg rat

> 4000 mg/kg rat

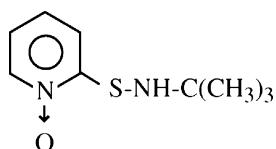
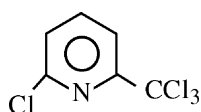
71 mg/kg rat

Antimicrobial effectiveness/application

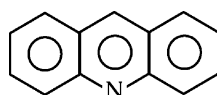
The brominated Hexamidine disposes of higher efficacy than Hexamidine. Primarily the effectiveness is directed against bacteria, whereas the activity against moulds and yeasts is moderate. Optimal is a pH between 5 and 8. Dibromohexamidine is not compatible with anionic surfactants and proteins. In the EEC Cosmetic Directive it is listed under No. 15 as a preservative with a maximum permitted concentration of 0.1%.

13. Pyridine Derivatives and Related Compounds (Benzopyridines = Quinolines)

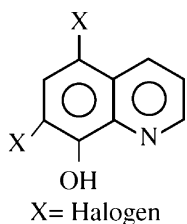
Among other pyridine derivatives 2-hydroxy-pyridine-*N*-oxides, 2-mercaptopyridine-*N*-oxides and 8-hydroxy-quinolines are described in this section which may be looked at as membrane-active microbicides with chelating properties. The following pyridine compounds with antimicrobial activity but without significant importance for the protection of materials shall only be mentioned:

2-(tert.-butylaminothio)pyridine-*N*-oxide

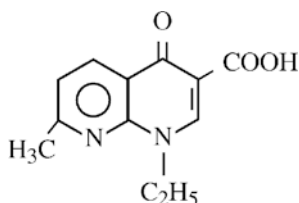
2-chloro-6-(trichloromethyl)pyridine – Nitrapyrin



dibenzpyridine – Acridine



Halo-8-hydroxyquinolines, especially 5,7-dihalo-8-hydroxyquinolines transgress the parent compound Oxine (13.3.) in antimicrobial activity



1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid – Nalidixic acid

Quaternary pyridinium and isoquinolinium salts are described under 18.1. 'Quaternary Ammonium Compounds'.

The pyridine derivative 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine appropriately is listed under 17. 'Compounds with Activated Halogen Atoms'.

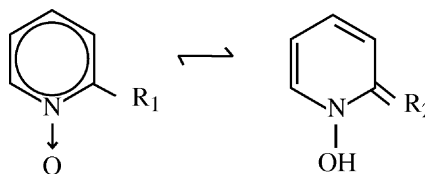
Microbicide group (substance class)

Chemical name

Structural formula

13. PYRIDINE DERIVATIVES AND RELATED COMPOUNDS

13.1. Pyridine-N-oxides



$R_1 = \text{OH}$: 2-Hydroxypyridine-N-oxide

$R_1 = \text{SH}$: 2-Mercaptopyridine-N-oxide

$R_2 = \text{O}$: 1-Hydroxy-(1H) pyridine-2-one

$R_2 = \text{S}$: 1-Hydroxy-(1H) pyridine-2-thione

The N-oxide and the N-hydroxy form are in a tautomeric equilibrium.

The most important compound is 2-mercapto-pyridine-N-oxide = Pyrithione (13.1.3.). According to Albert (1968) its mechanism of antimicrobial activity is based on chelation complex formation. But there are findings (Cooney & Felix, 1972; Chandler & Segel, 1978) which demonstrate that other modes of action are involved, too, for example, influence on ATP levels, nutrient transport, interference with protein synthesis. Pyrithiones are used to prevent biodeterioration in aqueous functional fluids, e.g. adhesives, latex paints, polymer emulsions, including cosmetics. Pyrithiones are used very successfully in metal working fluids for the control of fungi; here they may assist formaldehyde releasing compounds (3.), such as hexahydro-s-triazines, e.g. hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine (3.3.18.), which are especially toxic for bacteria (Rossmore et al., 1978). Another application of pyrithiones is in fuel storage to inhibit microbial growth in entrained water and the resulting microbial induced corrosion and sludge formation.

Microbicide group (substance class)

Chemical name

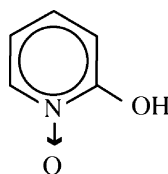
Chemical formula

Structural formula

13.1. PYRIDINE N-OXIDES

13.1.1. 2-Hydroxypyridine N-oxide

$\text{C}_5\text{H}_5\text{NO}_2$



Molecular mass	111.10
CAS-No.	13161-30-3
EC-No.	236-100-8
Synonym/common name	1-hydroxy-(1H)pyridine-2-one (tautomeric form), Oxy-Pyrrion
Supplier	PYRION-CHEMIE
Chemical and physical properties	
Appearance	white crystalline powder
Content (%)	~100
Melting point °C	151
Content (%) of a solution i. H ₂ O	20
Appearance	yellow-brown liquid
Density g/ml (20°C)	1.135
Refractive index	1.13885
pH	7–8
Stability (a. i.)	strong reducing agents attack the N-oxide configuration and reduce the compound to 2-hydroxypyridine
Solubility (a. i.)	soluble in H ₂ O, alkalis (salt formation) and alcohols
Toxicity data (a. i.)	
LD ₅₀ oral	920 mg/kg rat.
Irritant to skin and mucosa.	

Antimicrobial effectiveness/application

The microbicide may be used as a preservative for functional fluids.

Table 100 Minimum inhibition concentrations (MIC) of 2-hydroxypyridine-N-oxide (Source: PYRION-CHEMIE)

Test organism	MIC (mg/litre)
<i>Bacillus subtilis</i>	0.1
<i>Enterobacter aerogenes</i>	3.3
<i>Escherichia coli</i>	2.5
<i>Lactobacillus acidophilus</i>	0.2
<i>Proteus vulgaris</i>	1.8
<i>Pseudomonas aeruginosa</i>	3.5
<i>Serratia marcescens</i>	1.2
<i>Staphylococcus aureus</i>	0.4
<i>Streptococcus haemolyticus</i>	0.2

Microbicide group (substance class)

Chemical name

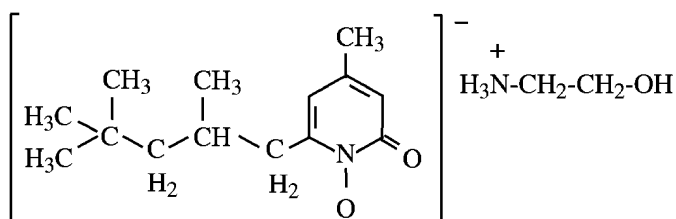
Chemical formula

Structural formula

13.1. PYRIDINE N-OXIDES

13.1.2. 1-Hydroxy-4-methyl-6-(2,4,2-trimethylpentyl)-2(1H)pyridone ethanolamine salt

C₁₆H₃₀N₂O₃



Molecular mass

298.46

CAS-No.

68890-66-4

EC-No.

272-574-2

EEC-No.

35

Synonym/common name	Piroctone olamine
Supplier	AVENTIS
Chemical and physical properties	
Appearance	white to slightly yellowish, odourless powder
Content (%)	~100
Melting point °C	136 (decomposition)
pH (1% in H ₂ O)	9–9.8
Stability	stable at pH 5 to 9 and temperatures up to 80°C;
Solubility g/l	~2 in H ₂ O, ~100 in alcohols, ~0.5–1 in oils, ~10–100 in water containing surfactants
Toxicity data	
LD ₅₀ oral	8100 mg/kg rat 5000 mg/kg mouse
No effect level for rats and dogs: 100 mg/kg/day.	
No sensitization.–No photo-toxic properties. –Non-teratogenic. –Non-mutagenic.	

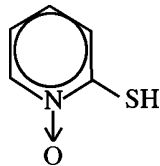
Antimicrobial effectiveness/application

Fungi and Pseudomonads are ten to twenty times more resistant to Piroctone olamine than other bacteria and yeasts. The main advantage of the microbicide is its low toxicity; it is practically non-toxic. As it is additionally compatible with anionic, cationic and amphoteric compounds it serves mainly as an active ingredient in antindandruff hair tonics, and as a preservative in shampoos. The EEC Cosmetic Directive authorizes the application of Piroctone olamine as a preservative with a maximum concentration of 1% for products rinsed off and 0.5% for other products.

As a preservative for other functional fluids Piroctone olamine has up to now not gained importance.

Table 101 Minimum inhibition concentrations (MIC) of Piroctone olamine in nutrient agar according to Wallhäuser (1984)

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	64
<i>Klebsiella pneumoniae</i>	32
<i>Pseudomonas aeruginosa</i>	625–1250
<i>Staphylococcus aureus</i>	32
<i>Candida albicans</i>	64
<i>Penicillium notatum</i>	625

Microbicide group (substance class)	13.1. PYRIDINE N-OXIDES
Chemical name	13.1.3. 2-Thiolpyridine N-oxide
Chemical formula	C ₅ H ₅ NOS
Structural formula	
Molecular mass	127.18
CAS-No.	1121-31-9
EC-No.	214-329-4
Synonym/common name	2-mercaptopyridine-1-oxide; tautomeric form: 1-hydroxy-(1H)pyridine-2-thione; Pyrithione
Supplier	SIGMA-ALDRICH

Chemical and physical properties

Melting point °C

Stability

69–72

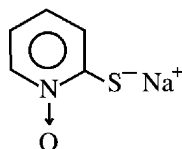
Pyrithione is very unstable in presence of oxygen or light at pH values < 4.5; strong reducing agents attack the N-oxide group; the reduction product is 2-thiopyridine which is less effective than the parent compound

Chemical name

Chemical formula

Structural formula

13.1.3a. 2-Thiopyridine N-oxide, sodium salt

 C_5H_4NNaOS 

Molecular mass

149.15

CAS-No.

3811-73-2

EC-No.

223-296-5

EPA TSCATS

Data base, Jan. 2001; EPA/FIFRA approval for antimicrobial applications

Synonym/common name

1-hydroxy-2(1H)pyridinethione, sodium salt (tautomeric form), Sodium Pyrithione

Supplier

ARCH, SIGMA-ALDRICH

Chemical and physical properties

Appearance

white to yellowish, crystalline powder with a faint odour

Content (%)

~100

Melting point °C

250 (decomposition)

pH (2% in H₂O)

8.0

Stability

in solution stable between pH 4.5 and 9.5; below pH 4.5 conversion to Pyrithione (13.1.3.); above pH 9.5 slow conversion to the salt of Pyrithione sulfonic acid; oxidizing agents such as peroxides and hypochlorites will convert Pyrithione first to 2,2'-dithio-bis(pyridine-N-oxide) (13.1.4.) and finally to inactive Pyrithione sulphinic or sulphonic acid (see Part I, Chapter 2, Figure 4); chelates spring up in contact with heavy metal ions > 500 in H₂O; > 150 in ethanol, > 100 in polyethylene glycol; moderately soluble in isopropanol (~8 g/l)

Solubility g/l

Toxicity data

LD₅₀ oral

870 mg/kg mouse

intraperitoneal

370 mg/kg mouse

subcutaneous

428 mg/kg mouse

intravenous

320 mg/kg mouse

Exposure limit (occupational)

Germany 1.0 mg/m³

Chemical and physical properties of an aqueous formulation

Appearance

yellow to amber liquid with a faint odour of pyridine

Content (%)

min. 40

Boiling point/range °C (101 kPa)

109

Solidification point °C

–25 to –30

Density g/ml (25°C)

1.27

Vapour pressure hPa (25°C)

25.27

Viscosity mPa·s (25°C)

10.98

Log POW

0.00015

pH (4% a. i. in H₂O)

8.5–10.5 at 25°C

Stability

heat resistant up to 100°C for at least 120 hours; precipitation of Pyrithione below pH 6.5; chemical reactivity as mentioned above for the active ingredient

Solubility	soluble in water and alcohol
Toxicity data (source: ARCH CHEMICALS INC.)	
LD ₅₀ oral	1500 mg/kg rat
LC ₅₀ on inhalation	2.7 mg/l for rats (4 h)
LD ₅₀ dermal	1800 mg/kg rabbit
Not known to be carcinogenic or mutagenic. Causes eye irritation; may cause irritation to skin, mucous membranes and respiratory tract.	
Ecotoxicity:	
LC ₅₀ for Bluegill sunfish	8.6 mg/l (96 h)
for Rainbow trout	7.3 µg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	22 µg a. i./l (48 h) (based on 100% a.i.)

Antimicrobial effectiveness/applications

Sodium Pyrithione is a widely used preservative for water based functional fluids. In consequence of its activity spectrum it is preferably applied when problems due to the growth of fungi have to be overcome, e.g. in metal working fluids. As is demonstrated by the MIC in Table 102 Sodium Pyrithione is a highly effective microbicide; the addition rates therefore are relatively low: 0.02–0.06%. However, users of Sodium Pyrithione have to pay attention to the fact that it is a chelating agent which in the presence of, for example, Fe²⁺ ions or Cu⁺ ions is converted to the corresponding chelates. These are sparingly soluble and highly coloured compounds. That means that they can cause colorations and precipitation, thus withdrawing active ingredients from the functional fluid to be protected. The ferric complex is blue, for example, and only a few ppm in a formulation can cause a noticeable discoloration. Between pH 7 and 9, chelating agents such as salts of diethylenetriaminepentaacetic acid or hydroxyethylene-diaminetriacetic acid are of some use at higher than theoretical amounts to inhibit coloration, if the chelating agent is added before the Sodium Pyrithione. The optimum scope of pH for the application of Sodium Pyrithione is between 7 and 10. Non-ionic detergents may partly inhibit the antimicrobial effectiveness of the compound.

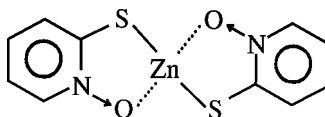
It is also recommended to use Sodium Pyrithion for the dry film protection of paints and adhesives. However in such applications one has to bear in mind in particular the colouration risk, the limited light stability and the leachability of the the active compound.

Table 102 Minimum inhibition concentrations (MIC) of Sodium Pyrithione in nutrient agar (Walläusser, 1984)

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	2
<i>Penicillium notatum</i>	2
<i>Trichophyton mentagrophytes</i>	0.5
<i>Candida albicans</i>	4
<i>Escherichia coli</i>	8
<i>Pseudomonas aeruginosa</i>	512
<i>Salmonella typhimurium</i>	64
<i>Staphylococcus aureus</i>	1
<i>Streptococcus faecalis</i>	2

Chemical name
Chemical formula
Structural formula

13.1.3b. Zinc-bis(2-thiopyridine-N-oxide)
C₁₀H₈N₂O₂S₂Zn



Molecular mass
CAS-No.
EC-No.
EPA/FIFRA
FDA

317.71
13463-41-7
236-671-3; EEC-No. 8
approval for antimicrobial applications
approval for use as an a. i. in antidandruff shampoo and hair dressing products

Synonym/common name	bis[1-hydroxy-2(1H)-pyridinethionato-O,S]-(T-4) zinc, Zinc Pyrithione
Supplier	ARCH CHEM. INC.
Chemical and physical properties	
Appearance	off-white crystalline powder of a mild odour
Content (%)	95–99
Melting point °C	240 (decomposition)
Bulk density g/ml	0.35
Vapour pressure hPa (25°C)	negligible
Auto ignition temperature °C	410
Log POW	0.93
pH (5% slurry in H ₂ O)	6.5–8.5
Stability	the chelated zinc complex is stable between pH 4 and 8.5; below pH 3.5 conversion to Pyrithion (13.1.3.); in alkaline solutions (pH > 8.5) conversion to soluble alkali salts (e.g. 13.1.3a); transchelation in the presence of heavy metal ions; even traces of the corresponding chelates can cause a noticeable colouration, foremost the iron and copper complex; sensitive to strong oxidizing and reducing agents and light (see 13.1.3. and 13.1.3a)
Solubility g/l (25°C)	0.008 in H ₂ O (pH 7), 0.1 in ethanol, 0.08 in isopropanol, 0.2 in propyleneglycol, 2 in polyethylene glycol, 3 in chloroform, 80 in diemthyl formamide, < 0.001 in oils
Toxicity data (source: ARCH CHEM. INC.)	
LD ₅₀ oral	269 mg/kg rat > 1000 mg/kg monkey
LD ₅₀ dermal	> 2000 mg/kg rabbit
Oral application for 28 days to monkeys: No Adverse Effect Level 11.0 mg/kg/day. Irritant to skin and mucous membranes; corrosive to eyes.	
Not known to be carcinogenic nor mutagenic. Exposure limit (occupational)/ARCH Internal	
Exposure Standard: TWA (8 h)	0.35 mg/m ³ .
Ecotoxicity:	
LC ₅₀ for Rainbow trout	3.2 µg/l (96 h)
Sheepshead minnow	400 µg/l (96 h)
Fathead minnow	2.6 µg/l (96 h)
EC ₅₀ for Daphnia magna	34 µg/l (48 h)
Mysid shrimp	6.3 µg/l (96 h)
Oyster shell deposition	22 µg/l (96 h)

Antimicrobial effectiveness/application

Zinc Pyrithione's spectrum of efficacy covers moulds, yeasts, bacteria and algae (see Table 103). It may be used as an in-can preservative for a great variety of aqueous formulations, including cosmetics. As Zinc Pyrithion is not volatile and of very low water solubility, it is also applied as a dry film preservative in paints (including marine antifoulant coatings), polymers, plastics, carpet fibers, adhesives, sealants and other materials. Exterior latex paints containing 2–3% Zinc Pyrithione (calculated on total wet paint) bring about long term mould and algae resistant paint films; in interior coatings the addition rates move between 1–2%. In antifouling marine paint combinations of Zinc Pyrithione (3–5% calcul. on dry film weight) and cuprous oxide (35–40%) are more effective than either biocide alone. However, the cheleated Zn-complex should not be used in alkyd or highly alkyd modified latex paints that contain metal carboxylate driers, as transchelation and colouration can occur.

As a preservative for the protection of cosmetic products Zinc Pyrithione is applied in concentrations in general varying between 0.025 and 0.1%. In the EEC Cosmetic Directive the microbicide is mentioned with a maximum authorized concentration of 0.5%, however, with the limitation authorized only in products rinsed off, forbidden in products for oral hygiene. Percentage of use in US cosmetic formulations: 0.14%.

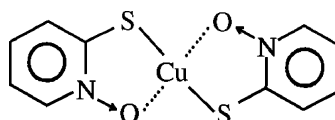
Table 103 Minimum inhibition concentrations (MIC) of Zinc Pyrithion dissolved in H₂O using dimethylsulphoxide as a cosolvent (Source: ARCH CHEM. INC.)—Cfu of bacteria 10⁶/ml and 10⁵/ml of fungal spores

Test organism	ATCC No.	MIC (mg/l)
Gram Positive Bacteria		
<i>Staphylococcus aureus</i>	6538	4
<i>Streptococcus faecalis</i>	19433	16
Gram Negative Bacteria		
<i>Escherichia colic</i>	9637	8
<i>Pseudomonas aeruginosa</i>	9721	512
<i>Klebsiella pneumoniae</i>	4352	8
Molds		
<i>Fusarium sp.</i>	—	32
<i>Aspergillus niger</i>	9642	8
<i>Aureobasidium pullulans</i>	9348	2
<i>Chaetomium globosum</i>	6205	2
<i>Gliocladium virens</i>	9645	64
<i>Penicillium pinophilum</i>	9644	2
Yeasts		
<i>Candida albicans</i>	11651	2
<i>Pityrosporum ovale</i>	—	4
Actinomycete		
<i>Streptoverticillium reticulum</i>	25607	4
Algae		
<i>Trentepohlia odorata</i>	—	≤0.06
<i>Anacystis montana</i>	—	≤0.06
<i>Chlorococcum tetrasporum</i>	—	8
<i>Scytonema hofmannii</i>	—	0.5
<i>Synechocystis minima</i>	—	≤0.06

Zinc Pyrithione is incompatible with EDTA; nonionic detergents can reduce its antimicrobial activity. pH 4–8 is optimal for the application of Zinc Pyrithione.

Chemical name
Chemical formula
Structural formula

13.1.3c. Copper-bis(2-thiopyridine-N-oxide)
C₁₀H₈N₂O₂S₂Cu



Molecular mass
CAS-No.
EC-No.
EPA-Reg.
Synonym/common name
Supplier

315.85
14915-37-8
238-984-0
in process; not listed on the TSCA inventory
Copper Pyrithione
ARCH CHEM./INC.

Chemical and physical properties

Appearance
Content (%)
Melting point °C
Bulk density g/ml
pH (5% slurry in H₂O)
Stability

olive green solid
~96
~282 (decomposition)
< 0.35
6.0–9.0
sensitive to strong oxidizing agents; direct exposure to UV radiation causes slow decomposition
< 1 mg/l in H₂O

Solubility

Toxicity data (source: ARCH CHEM. INC.)

LC₅₀ on inhalation
Copper Pyithione paste:
LD₅₀ (based on constituents) oral
dermal

0.14 mg/l (4 h, rat-nose-only)

1000–2000 mg/kg rat
> 2000 mg/kg rabbit

Copper Pyrithione is irritant to the skin, may cause severe eye irritation and sensitization.

Ecotoxicity of Copper pyrithione:

LC ₅₀ for fish (Fathead minnow)	0.0043 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	0.022 mg/l (48 h)
EC ₅₀ for freshwater algae	0.035 mg/l (120 h)

Antimicrobial effectiveness/application

Cooper Pyrithione disposes of strong algacidal activity and extremely low water solubility, basic requirements for an active ingredient in antifouling coatings with long lasting performance. Combinations of Copper Pyrithione and cuprous oxide are more effective than either biocide alone. Proposed addition rates: 3–5% Copper Pyrithione plus 30–40% Cuprous oxide (calculated on total weight of paint formulation).

Microbicide group (substance class)

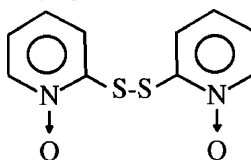
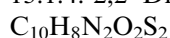
Chemical name

Chemical formula

Structural formula

13.1. PYRIDINE N-OXIDES

13.1.4. 2,2'-Dithio-bis(pyridine-N-oxide)



Molecular mass

252.32

CAS-No.

3696-28-4

EC-No.

223-024-5

Synonym/common name

bis(2-pyridyl-N-oxide)-disulphide, 2,2'-dithio-bis(pyridine-1,1'-dioxide), Dipyrithione, Pyrithione disulphide

Chemical and physical properties

Appearance

off-white to tan crystalline, slightly hygroscopic solid with a faint odour

Content (%)

~100

Melting point °C

205 (decomposition)

pH (~1% in H₂O)

approx. 7

Stability

stable in mineral or acetic acid solutions (formation of soluble acid salts without cleavage of the S–S bond); in alkaline solutions slow cleavage of the disulphide linkage under release of alkali salts of 2-mercapto pyridine N-oxide (e.g. 13.1.3a.) and to a certain extent Pyrithione sulphinate.

Solubility g/l (21°C)

11.3 in H₂O at pH 4.5, 8.1 in ethanol, 1.1 in acetone, 28.1 in chloroform, < 0.01 in hexane

Toxicity data

LD₅₀ oral

1640 mg/kg male rat

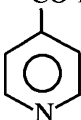
1240 mg/kg female rat

543 mg/kg mouse

Mildly irritant to the skin (test with rabbits); a 0.8% solution in water did not cause irritation on rabbit eyes.

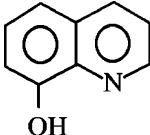
Antimicrobial effectiveness/applications

The antimicrobial activity of Pyrithione disulphide corresponds to the activity of Pyrithione (13.1.3.) which is released, when the disulphide bond splits up; that means also the disulphide is especially toxic for mould producing fungi and Gram-positive bacteria and not very effective against Gram-negative bacteria. It may be used for the in-can/in-tank protection of aqueous functional fluids including jet fuel. With the application of Pyrithione disulphide colouration risks, as described for sodium Pyrithione (13.1.3a.), have to be taken into consideration, too.

Microbicide group (substance class)	13. PYRIDINE DERIVATIVES AND RELATED COMPOUNDS
Chemical name	13.2. Pyridine-4-carboxylic acid hydrazide
Chemical formula	$C_6H_7N_3O$
Structural formula	$CO-NH-NH_2$ 
Molecular mass	137.14
CAS-No.	54-85-3
EC-No.	200-214-6
EPA TSCA	Section 8(B) Chemical Inventory
Synonym/common name	4-(hydrazinecarbonyl)pyridine, isonicotinic acid hydrazide, isonicotinoylhydrazine, Isoniazide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	≥99
Melting point °C	170–173
Stability	sensitive to oxidizing agents
Solubility	moderately soluble in water, soluble in polar solvents, virtually insoluble in ether, benzene
Toxicity data (selection)	
LD ₅₀ oral	1250 mg/kg rat 250 mg/kg rabbit 255 mg/kg guinea pig
LD ₅₀ intraperitoneal	335 mg/kg rat 147 mg/kg rabbit 195 mg/kg guinea pig
LD ₅₀ subcutaneous	329 mg/kg rat 135 mg/kg rabbit 195 mg/kg guinea pig
LD ₅₀ intravenous	365 mg/kg rat 94 mg/kg rabbit 220 mg/kg guinea pig
Irritant to skin, mucous membranes and eyes. Tumorigenic/carcinogenic by RTECS criteria.	

Antimicrobial effectiveness/application

Isoniazide is an amide derivative with a major potential for antimicrobial efficacy but also general toxicity, which is an obstacle to its practical application as a microbicide for the protection of materials. It has been used as a bacteriostat, primarily to inhibit the proliferation of tubercle bacteria, the cell wall of which is easily passed by Isoniazide molecules. Within the cytoplasm the isonicotinic acid hydrazide is split enzymatically to isonicotinic acid, which remains in the cell cytoplasm and as an antimetabolite of the cytoplasmic nicotinic acid disturbs the cell's metabolism.

Microbicide group (substance class)	13. PYRIDINE DERIVATIVES AND RELATED COMPOUNDS
Chemical name	13.3. 8-Quinolinol
Chemical formula	C_9H_7NO
Structural formula	

Molecular mass	145.16
CAS-No.	148-24-3
EC-No.	205-711-1
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	8-hydroxyquinoline, hydroxybenzopyridine, Oxine
Supplier	MERCK
Chemical and physical properties	
Appearance	colourless crystals or powder, almost odourless
Content (%)	~99
Boiling point/range °C (101 kPa)	267
Melting point °C	76
Stability	chelating agent (approx. 30 metal ions form with Oxine ionic chelates); light sensitive
Solubility g/l	0.52 in H ₂ O at 18°C; 3.6 in H ₂ O at 90°C; soluble in ethanol, acetone chloroform, benzene, forms water soluble salts with mineral acids, e.g. Oxine chloride, Oxine sulphate
Toxicity data	
LD ₅₀ oral	1200 mg/kg rat 20000 mg/kg mouse
LC ₅₀ on inhalation	>1210 mg/m ³ (6 h)
LD ₅₀ intraperitoneal	43 mg/kg mouse
subcutaneous	83.6 mg/kg mouse

Evaluations of Glocke et al. (1981) strengthen the suspicion that Oxine is mutagenic; it is classified as tumorigenic by RTECS criteria.

Irritant to skin and mucous membranes.

Ecotoxicity: Oxine is biodegradable (test performed to OECD Guideline 301 D); however, Oxine disposes of considerable aquatic toxicity.

Antimicrobial effectiveness/applications

The antimicrobial activity described here is only valid for 8-hydroxyquinoline and not for the seven other possible hydroxyquinolines which are widely inactive and do not, as does 8-hydroxyquinoline, chelate metal cations, e.g. copper, iron and zinc. Contrary to Oxine the corresponding chelation complexes, e.g. copper 8-hydroxyquinoline (13.3a.), possess considerable lipid solubility enabling the complex to pass through the cell membrane and then to dissociate into the toxic 8-hydroxyquinoline. However, according to the findings of Block (1983) the

Table 104 Minimum inhibition concentrations (MIC) of Oxine in nutrient agar

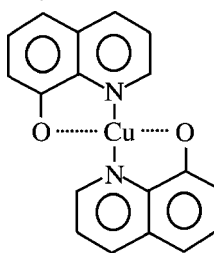
Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	5
<i>Aspergillus niger</i>	50
<i>Aureobasidium pullulans</i>	10
<i>Chaetomium globosum</i>	10
<i>Cladosporium cladosporioides</i>	5
<i>Coniophora puteana</i>	7.5
<i>Lentinus tigrinus</i>	10
<i>Penicillium glaucum</i>	10
<i>Polyporus versicolor</i>	20
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	50
<i>Aerobacter aerogenes</i>	15
<i>Aeromonas punctata</i>	15
<i>Bacillus mycoides</i>	35
<i>Bacillus subtilis</i>	7.5
<i>Escherichia coli</i>	100
<i>Leuconostoc mesenteroides</i>	75
<i>Proteus mirabilis</i>	100
<i>Pseudomonas aeruginosa</i>	500
<i>Pseudomonas fluorescens</i>	500
<i>Staphylococcus aureus</i>	20

chelation itself may already potentiate the anti-fungal activity of Oxine in addition to the resultant increase in lipid solubility. It is notable that Oxine copper is indeed around 100 times more effective than Oxine itself (see Part I, Chapter 2.).

As is demonstrated in Table 104 Oxine is a broad spectrum microbicide, although it is much more toxic for Gram-positive bacteria than for Gram-negative bacteria. But unfavourable solubility properties and coloration risks have prevented Oxine from becoming an important microbicide for material protection. More popular are the chelate complexes, above all copper 8-hydroxyquinoline.

Chemical name
Chemical formula
Structural formula

13.3a. Copper 8-quinolinolate
 $C_{18}H_{12}CuN_2O_2$



Molecular mass
CAS-No.
EC-No.
Synonym/common name

351.85
10380-28-6
233-841-9
copper 8-hydroxyquinoline, Oxine copper, 8-quinolinol copper salt
MERCK

Supplier

Chemical and physical properties

Appearance
Content (%)
Stability

crystalline, greenish, non-hygroscopic powder
~100 (copper content: 18)
stable between pH 3 and 12; light stable; heat resistant up to 270°C
insoluble in water, moderately soluble in xylene, trichloromethane; 0.4 in 1,2-dichlorobenzene

Toxicity data

LD₅₀ oral

4500 mg/kg rat

Antimicrobial effectiveness/application

Oxine copper is distinctively more effective than Oxine (13.3.) and a little bit more active than the corresponding zin chelate which offers, however, the advantage of being colourless. The activity spectrum of Oxine copper covers bacteria, yeasts, fungi and algae. Because of its high efficacy against wood staining and mould-producing fungi it is recommended for the protection of freshly sawn timber and wooden products, further for the protection of paper, cardboard, textile and plastic material, but only as far as the coloration caused through the incorporation of Oxine copper does not disturb. Another disadvantage of Oxine copper is the fact that the compound is not easily transferred into formulations appropriate for the homogeneous distribution of the active ingredient in the material to be protected from biodeterioration.

Table 105 Minimum inhibition concentrations (MIC) of Oxine copper in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus flavus</i>	2
<i>Aspergillus niger</i>	2
<i>Aureobasidium pullulans</i>	2
<i>Chaetomium globosum</i>	5
<i>Penicillium citrinum</i>	5
<i>Penicillium funiculosum</i>	2
<i>Trichoderma viride</i>	2

14. Azoles

Azole fungicides represent a new class of microbicides with favourable toxicological property patterns and low ecotoxicity; some of them are suitable for the fungicidal treatment of various materials, especially wood and polymers.

The antimicrobial action of the azole derivatives described in this section is based primarily on the inhibition of ergosterol biosynthesis, e.g. in fungi, through blocking the biosynthetic reactions in the conversion of lanosterol to ergosterol (Kato *et al.*, 1986). More precisely, the azole fungicides act as inhibitors for C-14 demethylase which is responsible for the 14- α -demethylation of lanosterol leading to ergosterol (Figure 18).

Azole derivatives with antifungal activity, e.g. those listed here, can be characterized as substituted aromatic heterocycles (imidazole, triazole) with an unsubstituted nitrogen atom at the *m*-position and one nitrogen atom bearing lipophilic groups including a benzene ring (see Figure 19). They are able to attack cytochrome P-450 involved in the 14- α -demethylation of lanosterol. The presence of the *m*-nitrogen atom is absolutely necessary for the antifungal activity, respectively, the inhibition of C-14 demethylase. It is likely that the *m*-N atom of the aromatic heterocycles binds coordinatively to the protohaem iron of cytochrome P-450. The lipophilic part of active azoles which is structurally variable to a great extent may bind to cytochrome P-450 at various positions through the formation of hydrophobic bonds and in some cases hydrogen bonds. As ergosterol is a principle sterol in fungi and an indispensable compound in the membrane structures, the azole fungicides affect membrane permeability; at lower inhibitor concentrations this ultimately results in retardation of fungal growth. At higher concentrations the azoles act fungicidally; they disrupt fungal membranes immediately, because they probably act as cationic surface active ingredients (membrane-active microbicides) (Kato *et al.*, 1986).

They were developed as fungicides for the protection of plants and seeds. Not only their comparatively high efficacy (which permits lower concentrations in application), but also their selectivity and generally very good environmental acceptance are undeniable assets. At first, however, the azole active ingredients were not regarded as interesting fungicides for the protection of materials because of their unbalanced effect spectrum (see Table 106). But in view of a demand for microbicides of very low toxicity and an activity spectrum graduated according to the intended use it became apparent that there were fields of application for which the azole fungicides' limited effect spectrum seemed to be tailored. That applies to the field of wood preservation: here the azole fungicides can perform partial functions, namely the control of wood-decaying fungi. Other fungicides which have also a limited effect spectrum but on the other hand an extremely high degree of efficacy, e.g. dichlofluanide (16.5.), are able to control the wood-staining fungi. And since a new generation of insecticides has also been developed, it is today possible to formulate wood preservatives on the basis of active substances of low toxicity and ecotoxicity. These modern wood preservatives are able to replace those of doubtful toxic and ecotoxic characteristics, especially formulations with pentachlorophenol or organotin compounds.

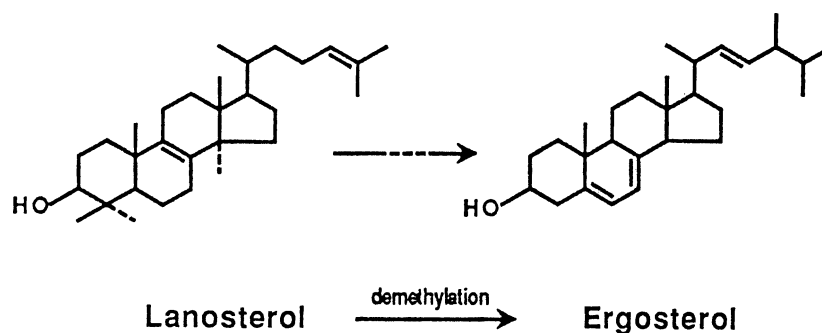


Figure 18 Demethylation of lanosterol to ergosterol.

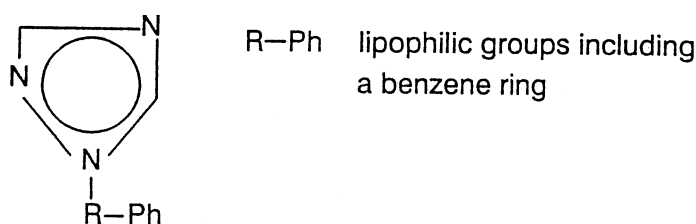


Figure 19 Azole derivatives with antifungal activity.

Table 106 Minimum inhibition concentrations (MIC) (mg/litre) of three azole fungicides in nutrient agar

Test organism	Tebuconazole*	Azaconazole**	Propiconazole***
<i>Alternaria alternata</i>	200	1000	350
<i>Aspergillus niger</i>	10	250	100
<i>Aureobasidium pullulans</i>	35	35	5
<i>Cephalosporium fragrans</i>	5	25	5
<i>Ceratocystis pilifera</i>	20	50	10
<i>Chaetomium globosum</i>	15	75	10
<i>Cladosporium cladosporioides</i>	50	35	
<i>Glomerella virens</i>	75	350	35
<i>Lentinus tigrinus</i>	10	50	10
<i>Penicillium glaucum</i>	15	200	100
<i>Phanerochaete sanguinea</i>	10	50	
<i>Phialophora fastigiata</i>	50	1000	100
<i>Sclerophoma pityophila</i>	5	5	1
<i>Stereum sanguinolentum</i>	5	15	
<i>Trichoderma viride</i>	> 1000	> 1000	> 1000

*14.1. **14.3. ***14.2.

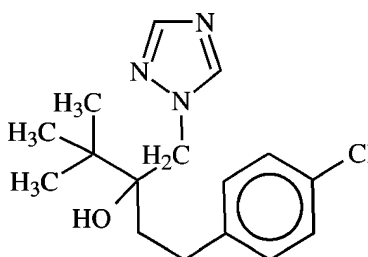
Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

14. AZOLES

14.1. α -[2-(4-Chlorophenyl)ethyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazolyl-(1)-ethanolC₁₆H₂₂ClN₃O

Molecular mass

307.83

CAS-No.

107534-96-3

EC-No.

403-640-2

JAPAN

MITI and MOL reg. as new substance

EPA Reg.

approval for antimicrobial applications

Synonym/common name

 α -tert Butyl- α -(p-chlorophenylethyl)-1H-1,2,4-triazole-1-ethanol, Tebuconazole

Supplier

BAYER

Chemical and physical properties

Appearance

colourless to beige, odourless crystals

Content (%)

~95

Melting point °C

approx. 105

Density g/ml (20°C)

approx. 1.25

Vapour pressure hPa (20°C)

 1.7×10^{-8} (at 60°C: 6×10^{-6})Bulk density kg/m³

approx. 400

Flash point °C

> 185

Ignition temperature °C

approx. 468

Log POW

3.7

PH (~10% aqueous dispersion)

6.0

Stability

comparatively heat resistant; stable in acidic up to strong alkaline formulations

Solubility g/l (20°C)

0.036 in H₂O, ~270 in ethylene glycol, ~255 in propylene glycol monomethyl ether, ~215 in dipropylene glycol monomethyl ether, ~29 in Solvesso 100, ~3 in Crystalol 60, ~3 in white spirit

Toxicity data (source: BAYER)

LD₅₀ oral

approx. 4000 mg/kg male rat

1700 mg/kg female rat
 LC₅₀ dermal > 5000 mg/kg rat
 LD₅₀ on inhalation (dust) > 5000 mg/m³ air (4 h) for rats
 In tests with rabbits Tebuconazole proved to be non-irritant to skin and eyes.

Ecotoxicity:

Tebuconazole is classified "not readily degradable" (approx. 20% in tests according to OECD-Test-Guideline 301 C; method of analysis: BOD and chloride determination).

LC₅₀ for fish: *Lepomis macrochirus* 5.7 mg/l (96 h)
Oncorhynchus mykiss 4.4 mg/l (96 h)
 EC₅₀ for *Daphnia magna* 4.2 mg/l (48 h)
 IrC₅₀ for green algae: *Scenedesmus subspicatus* 4.01 mg/l (96 h; r = growth rate)
 EC₅₀ for activated sludge organisms > 10000 mg/l

Antimicrobial effectiveness/applications

Tebuconazole is a potent fungicide. However, there are some gaps in its activity spectrum. Together with other microbicides, filling the gaps in the spectrum of effectiveness (see Table 106), Tebuconazole may be used for the fungicidal treatment of materials as described by Büchel et al. (1988). Evaluating a test report of Gründlinger & Exner (1990) it will be noticed that Tebuconazole is particularly effective against wood decaying fungi (Basidiomycetes), but less effective against blue-staining fungi. Remarkable is also the *Trichoderma* gap. In aqueous wood preservatives or paint film fungicides for aqueous surface coatings the gaps mentioned may be filled for instance with Carbendazim (11.4.). In solvent based wood preservatives and paints it is recommended to apply Tebuconazole together with blue stain fungicides, such as Dichlofluanide (16.5.) or Tolyfluanide (16.6.). Tebuconazole is also compatible with insecticides, e.g. with Cyfluthrin. Application concentrations move between 0.4–1.5% Tebuconazole.

Tebuconazole is un-leachable, heat-resistant, non-volatile, stable in acid and alkaline media and accordingly suitable for long-term protection of wood and wood-based products, of paint films, plastics, sealants, adhesives etc., The resistance of Tebuconazole to weathering is demonstrated by the toxic values for fungi in a soil block test:

Soil block test AWP A E10-91 carried out by test institute MSFPL*

Test fungus	Wood species	Weathering	Toxic values kg/m ³
<i>Coriolus versicolor</i>	Sweetgum	without	< 0.540
		with	< 0.546
<i>Irpex lacteus</i>	Sweetgum	without	0.018–0.019
		with	0.019–0.021
<i>Gloeophyllum trabeum</i>	Ponderosa pine	without	0.018–0.0193
		with	0.026–0.030
<i>Poria placenta</i>	Ponderosa pine	without	0.117–0.123
		with	0.138–0.144

*MSFPL = Mississippi State Forest Products Laboratory, USA

Microbicide group (substance class)

Chemical name

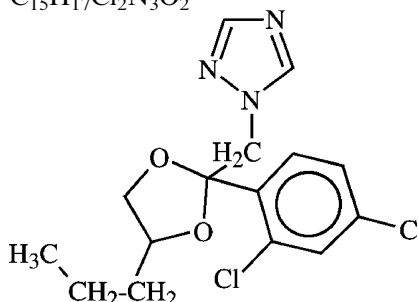
Chemical formula

Structural formula

14. AZOLES

14.2. 1-[(2-(2',4'-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole

C₁₅H₁₇Cl₂N₃O₂



Molecular mass

342.24

CAS-No.	60207-90-1
EC-No.	262-104-4
EPA Reg.	approval for antimicrobial applications
Synonym/common name	Propiconazole
Supplier	JANSSEN PHARMACEUTICA
Chemical and physical properties	
Appearance	light-yellow to dark-yellow liquid of high viscosity and a slight characteristic smell
Content (%)	> 93
Boiling point/range °C (101 kPa)	> 250; (180 at 0.013 kPa)
Density g/ml (20°C)	~1.289
Vapour pressure hPa (20°C)	2.7×10^{-7} (5.6×10^{-7} at 30°C)
Viscosity mPa·s (20°C)	69630 (3509 at 40°C)
Refractive index nD (20°C)	1.547
Flash point °C	approx. 200
Ignition temperature °C	approx. 430
Log P hexane/water (25°C)	3.72
pH (1% i H ₂ O at 25°C)	4.9
Stability	stable in acid and alkaline solution; heat- and light-stable
Solubility g/l (20°C)	~0.1 in H ₂ O, > 500 in 2-propanol, > 500 in propylene glycol, 260 in ethylene glycol, > 100 in white sprit, ~47 in hexane
Toxicity data (source: BAYER)	
LD ₅₀ oral	1517 mg/kg rat 1490 mg/kg mouse
LC ₅₀ on inhalation	> 5800 mg/m ³ (aerosol, exposure 4 h)
LC ₅₀ dermal	> 4000 mg/kg rat > 6000 mg/kg rabbit

Propiconazole is classified as non-mutagenic and non-teratogenic.

In tests with rabbits the microbicide proved to be non-irritant to the skin and mucous membranes. However, it may cause sensitization by skin contact.

Ecotoxicity: Water quality is highly impaired by Propiconazole.

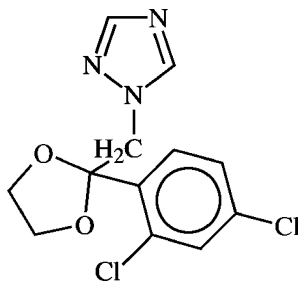
LC ₅₀ for fish: Rainbow trout	5.3 mg/l (96 h)
Cyprinus carpio	6.8 mg/l (96/h)
Lepomis macrochirus	6.4 mg/l (96 h)
LC ₅₀ for Daphnia magna	10.2 mg/l (48 h)
IrC ₅₀ for algae (Scenedesmus subs.):	0.76 mg/l (72 h; r = growth rate)

Antimicrobial effectiveness/applications

The activity spectrum of Propiconazole which is demonstrated by the MICs in Table 106, is similar to that of Tebuconazole (14.1.). Although Propiconazole shows a somewhat better water solubility and higher vapour pressure than Tebuconazole it may according to extensive tests performed by Valcke (1989) be regarded as a most interesting wood preserving fungicide, being resistant to leaching and evaporation stresses and having as good stability in treated wood and in formulations. A more thorough analysis of the test results (Gründlinger & Exner, 1990; Valcke, 1989) discloses that Tebuconazole and Propiconazole differ in their effect on differing wood decaying fungi but complement each other: Tebuconazole is more effective against Gloeophyllum trabeum and Propiconazole more effective against species of Poria. The proposals, to applicate Tebuconazole together with blue stain fungicides, are also valid for Propiconazole. 0.6–1.5% Propiconazole are recommended as application concentrations.

Microbicide group (substance class)	14. AZOLES
Chemical name	14.3. 1-[2-(2,4-Dichlorophenyl)-1,3-dioxolan-2-yl-methyl]-1H-1,2,4-triazole

Chemical formula
Structural formula



Molecular mass
CAS-No.
EC-No.
Synonym/common name
Supplier

300.14
60207-31-0
262-102-3
Azaconazole
JANSSEN PHARMACEUTICA

Chemical and physical properties

Appearance white to brownish powder
Content (%) > 95
Boiling point/range °C (101 kPa) 450
Melting point °C 100–110
Vapour pressure hPa (20°C) 5.3×10^{-9} (2.7×10^{-6} at 70°C)
Stability heat resistant up to 220°C; stable in acid and alkaline solutions (pH 1–9) at storage temperatures up to 40°C
Solubility g/l 0.3 in H₂O, 158 in methanol, 36 in n-propanol, 35 in ethylene glycol, 66 in propylene glycol, 156 in acetone, 111 in toluene, 45 in xylene, 345 in cyclohexanone

Toxicity data (source: JANSSEN PHARMACEUTICA)

LD₅₀ oral 308 mg/kg rat
1123 mg/kg mouse
LC₅₀ dermal > 2500 mg/kg rat

Moderately irritant to skin and mucosa.

In sub-chronic, chronic, oncogenicity and reproduction studies performed in rats, mice, dogs and rabbits, Azaconazole proved to be a very safe chemical. Several mutagenicity tests conducted in different biological systems demonstrated the lack of genetic effects.

Ecotoxicity:

LC₅₀ for Bluegill sunfish 18–23 mg/l (96 h)
Brown trout 22 mg/l (96 h)
LC₅₀ for Daphnia magna 86 mg/l (48 h)

Antimicrobial effectiveness/applications

According to Valcke & Goodwine (1985) Azaconazole is effective against Basidiomycetes and staining fungi (blue stain, sapstain and mould species). Because of its stability Azaconazole may be formulated in aqueous and solvent based wood preservatives or into emulsion concentrates to be used for solid wood and modified wood out of ground contact. However, in the meantime the other azole derivatives, Tebuconazole (14.1.) and Propiconazole (14.2.) are regarded as the more interesting fungicides for use in wood preservatives.

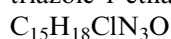
Microbicide group (substance class)

14. AZOLES

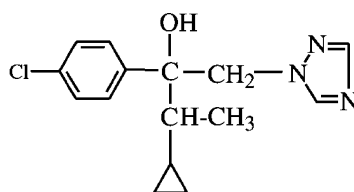
Chemical name

14.4. α -(4-Chlorophenyl)- α -(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

Chemical formula



Structural formula



Molecular mass	291.8
CAS-No.	94361-06-5
EC-No.	not yet applicable
Synonym/common name	2-(4-chlorophenyl)-3-cyclopropyl-(1H-1,2,4-triazol-1-yl)-2-butanol, Cyproconazole
Supplier	BAYER CROP SCIENCE, JANSSEN PHARMACEUTICAL, NOVARTIS

Chemical and physical properties

Appearance	light beige to brownish crystalline powder
Content (%)	≥94
Melting point °C	106.2–106.9
Density g/ml (21°C)	1.25
Vapour pressure hPa (25°C)	0.003
Surface tension mN/m (20°C)	65.2 (OECD 115)
pH (1% in H ₂ O at 20°C)	approx. 7
Log POW (25°C)	3.1
Stability	resistant to hydrolysis in acidic to strong alkaline media; heat-resistant; no photo-degradation at long term outdoor exposure; not leachable and non volatile
Solubility g/l (25°C)	0.14 in H ₂ O; > 200 in methanol, 109 in toluene, > 200 in acetone

Toxicity data (source: BAYER CROP SCIENCE)

LD ₅₀ oral	1115–1342 mg/kg rat
LD ₅₀ dermal	> 2000 mg/kg rabbit
LC ₅₀ inhalative for rats	> 5.65 mg/l (4 h)

Not irritant to skin and mucous membranes. No sensitizing effect was observed in the guinea-pig (Magnusson-Kligman test).

No mutagenic effects were observed in various in-vivo and in-vitro investigations. Toxic doses administered to pregnant female rats resulted in foetal defects.

Ecotoxicity:

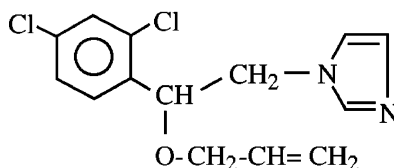
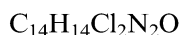
LD ₅₀ for Rainbow trout	19 mg/l (96 h)
EC ₅₀ for Daphnia magna	26 mg/l (48 h)
EC ₅₀ (biomass) for green alga (Selenastrum capricornutum)	0.01 mg/l (72 h)
EC ₅₀ (growth rate) of green alga (Desmodesmus subsp.)	0.077 mg/l (96 h)

Antimicrobial effectiveness/applications

The ergosterol biosynthesis inhibitor Cyproconazole has been developed by SANDOZ and is worldwide registered for use in agriculture. However the azole derivative shows also excellent efficacy against wood decaying and woodstaining fungi and against moulds. It is suitable for use as an active ingredient in solvent based and water based wood preservatives and antiblue stain agents. For surface applications concentrations of 0.1–0.4% are recommended. 0.04–0.38 kg a. i./m³ should be achieved in impregnation processes. Wood decaying fungi (basidiomycetes) are controlled at toxic values varying between 20–96 g a. i./m³ (EN 113). In EN 113 + EN 73 tests with aqueous concentrates of Cyproconazole toxic values of 70–100 a. i./m³ were found (source: JANSSEN).

Microbicide group (substance class)	14. AZOLES
Chemical name	14.5. 1-[2-(2,4-Dichlorophenyl)-2-(2-propenyloxy)ethyl]-imidazole

Chemical formula
Structural formula



Molecular mass

297.17

CAS-No.

35554-44-0

EC-No.

252-615-0

EPA TSCATS

Data base, Jan. 2001

Synonym/common name

Imazalil, 1-(β -allyloxy)-2,4-dichlorophenetyl)-imidazole

Supplier

RIEDEL DE HAEN

Chemical and physical properties

Appearance

slightly yellow to brown solidified oil

Content (%)

~98

Boiling point/range °C (101 kPa)

367

Melting point °C

50

Density g/ml (23°C)

1.243

Vapour pressure hPa (20°C)

9.3×10^{-5}

Refractive index n_D (20°C)

1.5643

Stability

tolerates temperatures up to 285°C; stable in dilute acids and alkalis at room temperature in the absence of light approx. 1 in H₂O; soluble in organic solvents

Solubility g/l

Toxicity data

LD₅₀ oral

227 mg/kg rat
> 640 mg/kg dog

LC₅₀ intraperitoneal

155 mg/kg rat

LC₅₀ dermal

4200 mg/kg rat and rabbit

LC₅₀ on inhalation

16 g/m³ (4h) for rats

No indication of teratogenicity in rats.

Irritant to skin and mucosa; can cause irreversible eye damage.

Toxic to aquatic animals.

Antimicrobial effectiveness/application

The azole fungicide Imazalil is described here for completeness sake. It has been used as a systemic active plant protection fungicide and for the protection of citrus fruits against mould infestation.

Table 107 Minimum inhibition concentrations (MIC) of Imazalil in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	50
<i>Aspergillus niger</i>	50
<i>Aureobasidium pullulans</i>	5
<i>Chaetomium globosum</i>	10
<i>Cladosporium cladosporioides</i>	50
<i>Coniophora puteana</i>	50
<i>Lentinis tigrinus</i>	50
<i>Penicillium digitatum</i>	1
<i>Penicillium glaucum</i>	100
<i>Penicillium italicum</i>	1
<i>Polyporus versicolor</i>	100
<i>Rhizopus nigricans</i>	200
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	500

15 Heterocyclic N, S compounds

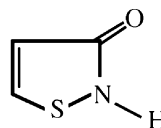
Meant are cyclic organic microbicides containing N and S atoms in pseudoaromatic ring systems, e.g. thiazole or isothiazole derivatives.



Thiazole



Isothiazole



4-Isothiazolin-3-one

The saturated heterocyclic N, S compounds Dazomet (3.3.25.) and Taurolidine (3.5.2.) result from condensation reactions of formaldehyde (2.1.) with amino groups and accordingly are listed under 3. Formaldehyde Releasing Compounds., as well as N-hydroxymethyl-2-mercaptobenzthiazole which is obtained by the addition of formaldehyde to the heterocyclic N, S compound 2-mercaptobenzthiazole (15.10.).

Microbicide group (substance class)

Chemical name

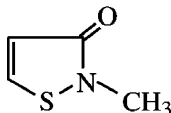
Chemical formula

Structural formula

15. HETEROCYCLIC N,S COMPOUNDS

15.1. 2-Methyl-4-isothiazolin-3-one (MI)

C₄H₅NOS



Molecular mass

115.16

CAS-No.

2682-20-4

EC-No.

220-239-6

EPA Reg.

application filed

MITI (Japan)

listed

Synonym/common name

2-methyl-3(2H)-isothiazolone

Supplier

ROHM AND HAAS, THOR

Chemical and physical properties of an aqueous solution

Appearance

colourless to pale yellow liquid with a mild odour

Content

~50 in water

Boiling point/range °C (101 kPa)

100

Density g/ml (20°C)

1.2

Vapour pressure hPa (20°C)

0.083

pH (as is)

4-5

Stability

stable in the presence of light, over the pH range 2-10 and up to 80°C; sensitive to oxidizing and reducing agents; as an electrophilic active agent MI is susceptible to nucleophilic attack, e.g. by amines and mercaptans infinitely soluble in H₂O; miscible in a wide range of water soluble organic solvents

Solubility

Chemical and physical properties of the a. i. (~100%)

Appearance

colourless, extremely hygroscopic crystals

Boiling point °C (0.004 kPa)

93

Melting point °C

50-51

Vapour pressure hPa

5.85×10^{-5}

Log POW

0.5

Stability

at exposure to air conversion into an oily compound; decomposition on heating starts at 55°C

Solubility g/l

30 in H₂O; highly soluble in organic solvents

Toxicity data (source :THOR)

LD ₅₀ oral	285 mg/kg rat
dermal	> 2000 mg/kg rat

Caustic effect on skin and mucous membranes, can cause severe eye corrosion. Sensitization by skin contact is possible.

Ecotoxicity (source: ROHM AND HASS):

Abiotic degradation	hydrolysis: half-life at pH 5 > 720 h at pH 7 > 720 h at pH 9 > 720 h photolysis: half-life at pH 7 266 h
Biotic degradation	half life in an aerobic aquatic microcosm: 9 h

The pathway for biodegradation of MI-cleavage of the activated N-S bond and further reactions – is discussed in Part I, Chapter 2.

The log POW for MI indicates that the microbicide has a negligible potential for bioaccumulation.

Antimicrobial effectiveness/applications

The MIC's indicated in Table 108 show that MI disposes of broad spectrum of effectiveness. Most sensitive react bacteria. The water soluble MI concentrate is easy to apply; the active ingredient is stable in high pH formulations, stays mainly in the aqueous phase where biological control is needed. Accordingly the MI concentrate is suitable for the wet-state preservation of a wide range of aqueous formulations. The addition rates move between 0.05 to 0.15%.

Table 108 Minimum inhibition concentrations (MIC) in mg/l of 2-methyl-4-isothiazoline-3-one for relevant spoilage organisms (Source: THOR)

Bacteria	MIC	Moulds	MIC	Yeasts	MIC
Escherichia coil	17.5	Aspergillus niger	750	Candida valida	75
Klebsiella pneumoniae	20.0	Paecilomyces variotii	100	Saccharomyces cerevisiae	150
Proteus vulgaris	25.0	Penicillium funiculosum	200		
Pseudomonas aeruginosa	30.0				
Pseudomonas putida	12.5				
Pseudomonas stutzeri	12.5				

Microbicide group (substance class)

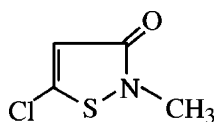
Chemical name

Chemical formula

Structural formula

15. HETEROCYCLIC N,S COMPOUNDS

15.2. 5-Chloro-2-methyl-4-isothiazolin-3-one (CMI)

C₄H₄ClNOS

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

149.60

26172-55-4

247-500-7

data base, Jan. 2001

5-chloro-2-methyl-3(2H)-isothiazolone

ROHM AND HAAS, SIGMA-ALDRICH

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Vapour pressure hPa (20°C)

Log POW

slightly yellow crystals

~100

54–55

0.0036

0.4

Stability	decomposition on heating starts at 55°C; sensitive to sodium bisulphite, SH compounds, amines, alkaline solutions (pH > 8)
Solubility g/l	approx. 5 in H ₂ O; moderately soluble in organic solvents
Toxicity data	
Acute toxicity see 15.3.	
Corrosive to skin, mucous membranes and eyes. Skin sensitizer.	
Ecotoxicity (source: ROHM AND HAAS):	
Abiotic degradation	hydrolysis: half-life at pH 5 > 720 h at pH 7 > 72 h at pH 9 528 h photolysis: half-life at pH 7 158 h
Biotic degradation	half-life in an aerobic aquatic microcosm 17 h
CMI is inherently biodegradable by micro-organisms. The pathway for biodegradation is discussed in Part I, Chapter 2. In tests performed with Bluegill sunfish a bioaccumulation factor of 5 has been determined which is in line with the log POW of CMI and indicates a minimal effect on aquatic organism and minimal potential to concentrate in the food chain.	

Antimicrobial effectiveness/application

In comparison to 2-methyl-4-isothiazolin-3-one (15.1.), the antimicrobial activity of which bases merely on the availability of an activated N-S bond susceptible to nucleophilic attack, the 5-chloro-2-methyl-4-isothiazolin-3-one disposes additionally of a vinyl activated chloro atom and therefore can be characterized as a molecule with two toxophoric structural elements (Paulus, 1988). As a result CMI should exhibit stronger antimicrobial efficacy than the halogen-free MI. This is confirmed by examinations of Diehl and Chapman (1999); see Table 109. Applications for CMI are described under 15.3.

Table 109 Minimum inhibition concentrations (MIC in mg/l) of CMI and MI determined using an inoculum of 5×10^6 cfu/ml

Test organism	ATCC	CMI	MI
<i>Pseudomonas aeruginosa</i>	15442	0.63 + / - 0.04	16.0. + / - 1.56
<i>Pseudomonas aeruginosa</i>	13388	0.43 + / - 0.03	13.8 + / - 1.46
<i>Pseudomonas fluorescens</i>	13525	0.20 + / - 0.02	17.3 + / - 2.9

Microbicide group (substance class)	15. HETEROCYCLIC N, S COMPOUNDS
Chemical name	15.3. Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one (15.2.) and 2-methyl-4-isothiazolin-3-one (15.1.)
CAS-No.	26172-55 + 2682-20-4
EC-No.	247-500-7 + 220-239-6; EEC-no: 39
EPA-Reg.	approval for antimicrobial application
FDA	various approvals
Supplier	ROHM AND HASS, THOR
Chemical and physical properties	
Composition	CMI (15.2.) 10–12%; MI (15.1.) 3–5%; magnesium nitrate 14–18%; magnesium chloride 8–10%; H ₂ O 60–64%
Such mixtures of 2-alkyl-4-isothiazolin-3-ones are formed in high yield by cyclization of N-alkyl-3,3'-dithiopropionamide induced through chlorine or sulphurylchloride (Lewis et al., 1971).	
Appearance	amber liquid with a pungent odour
Boiling point °C (101 kPa)	100
Solidification point °C	–33
Density g/ml	1.3

Viscosity mPa·s (25°C)	16
pH	1.0 to 3.0
Stability	decomposition starts at 50°C; aqueous functional fluids to be protected should be in the pH 4 to 8 range; water soluble Cu^{2+} salts may enhance the stability of the active ingredients up to a pH of 9; heat resistance is considerably increased in the presence of phenoxyalkanol, e.g. 1-phenoxy-propan-2-ol (1.9.) (Willingham & Mattox, 1990); sensitive to reducing agents (bisulphites, sulphides, mercaptans) and oxidizing agents; according to nature the electrophilic active ingredients may be deactivated by nucleophiles (e.g. primary and secondary amines) as long as these are not protonated (at pH values ≥ 7)
Solubility	completely soluble in H_2O and water soluble alcohols
Toxicity data (source: ROHM AND HAAS)	
LD ₅₀ oral	457 mg/kg rat
dermal	660 mg/kg rabbit
LC ₅₀ on inhalation for rats	0.33 mg/l a.i. (exposure: 4 h)

In tests with rabbits the product proved to be corrosive to skin and eyes. Sensitization: The product is classified as a skin sensitizer.

The product is neither carcinogenic nor mutagenic, nor teratogenic.

Ecotoxicity:

biodegradation (aquatic metabolism):

half-life of 15.2. under anaerobic conditions = 4.8 h

under aerobic conditions = 17.3 h

half-life of 15.1. under aerobic conditions = 9.1 h

LC₅₀ for Rainbow trout 0.19 mg a.i./l (96 h)

for Bluegill sunfish 0.28 mg a.i./l (96 h)

EC₅₀ for *Daphnia magna* 0.16 mg a.i./l (48 h)

EC₅₀ for algae (*Selenastrum*) 0.018 mg a.i./l

(*Skeletonema*) 0.003 mg a.i./l

Activated sludge respiration inhibition: EC₅₀ = 4.5 mg a.i./l

Antimicrobial effectiveness/applications

The mixing ration of the isothiazolidinones in the mixture described here is an optimum with regard to the antimicrobial effectiveness; higher concentrations of the non-chlorinated compound in such mixtures decrease the antimicrobial activity considerably. The combination 15.3. can be really considered as a broad spectrum microbistat, as is demonstrated by the MIC's listed in Table 110. There is no significant difference in activity against Gram-positive and Gram-negative bacteria. The algistatic concentrations for green and blue-green algae, and diatoms vary between 0.1 and 1.0 mg/l (ppm) a.i.. At concentrations 5–10 times higher than the microbistatic concentrations the mixture of active ingredients exhibits also microbicidal effectiveness if only slowly (incubation period 24 h).

Regarding the antimicrobial activity it is not surprising that the mixture of isothiazolinones has become one of the most important preservatives for the in-can/in-tank protection of aqueous functional fluids, including detergents and cosmetics. A big advantage of the active ingredient mixture is the fact that it does not show a *Pseudomonas* gap.

The recommended use levels move between 5 and 50 ppm (mg/l) a.i., corresponding to approximately 30–300 ppm of the formulation.

With regard to the sensitization potential of mainly CMI the EEC Cosmetics Directive has fixed a maximum authorized concentrations of 0.0015% (15 ppm) a.i. for the application of the CMI/MI 3:1 mixture as a preservative in cosmetic products. This concentration is also ruled as safe by the US Expert Panel of The Cosmetics Ingredients Review (C. I. R., 1990); a limit of 7.5 ppm is recommended in leave-on applications. In Japan 15 ppm ppm a.i. are permitted for rinse-off products.

As explained the isothiazolinones are of limited stability under certain conditions; it is therefore recommendable to check the effectiveness of the microbicides and the duration of their effect in finished formulations (see Part I, Chapter 2).

Table 110 Minimum inhibition concentrations (MIC) of the isothiazolinone formulation 15.3 (Source: LONZA)

Test organisms	ATCC-No.	MIC (mg a.i./l)
Fungi		
<i>Aspergillus niger</i>	9642	9
<i>Aspergillus oryzae</i>	10196	11.5
<i>Chaetomium globosum</i>	6205	9.0
<i>Glucocladium fimbriatum</i>	32913	4.5
<i>Mucor rouxii</i>	24905	9.0
<i>Penicillium funiculosum</i>	9644	11.5
<i>Pullularia (Aureobasidium) pullulans</i>	9348	2.3
<i>Rhizopus stolonifer</i>	10404	11.5
<i>Trichophyton mentagrophytes</i>	9533	4.5
Yeast		
<i>Candida albicans</i>	11651	9.0
<i>Rhototorula rubra</i>	9449	9.0
<i>Saccharomyces cerevisiae</i>	2601	9.0
Bacteria		
Gram positive		
<i>Brevibacterium ammoniagenes</i>	6871	9.0
<i>Bacillus cereus</i>	11778	9.0
<i>Bacillus subtilis</i>	6633	9.0
<i>Sarcina lutea</i>	9341	9.0
<i>Staphylococcus aureus</i>	6538	11.5
<i>Staphylococcus epidermidis</i>	155	9.0
<i>Staphylococcus agalactiae</i>	624	9.0
Gram negative		
<i>Achromobacter parvulus</i>	4335	4.5
<i>Alcaligenes faecalis</i>	8750	4.5
<i>Enterobacter aerogenes</i>	3906	9.0
<i>Escherichia coli</i>	11229	9.0
<i>Flavobacterium suaveolens</i>	958	9.0
<i>Klebsiella pneumoniae</i>	13883	9.0
<i>Proteus vulgaris</i>	8427	9.0
<i>Pseudomonas aeruginosa</i>	15442	9.0
<i>Pseudomonas cepacia</i>	25416	9.0
<i>Pseudomonas fluorescens</i>	13525	9.0
<i>Pseudomonas oleoverans</i>	8062	4.5
<i>Salmonella choleraesuis (typhi)</i>	6539	9.0
<i>Shighella sonnei</i>	9290	9.0
<i>Serratia marcescens</i>	8100	9.0

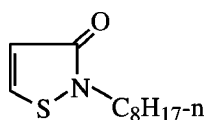
Worthy of note are findings of Riha et al. (1990), who observed that the use of Cu^{2+} ions together with CMI may effectively reduce the required dose of CMI and minimize its toxicological potential; apparently Cu^{2+} protects CMI from nucleophilic attack in the extracellular environment and additionally synergistically enhances its activity at the cellular level. The compatibility of the isothiazolinone preservative with non-ionic, anionic and cationic compounds is faced with adaptation/resistance development which has been demonstrated by Orth & Lutes (1985) for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Sondossi et al. (1999) isolated from a contaminated metalworking fluid a *Pseudomonas aeruginosa* strain which under laboratory conditions developed a positive function resistance to 200 µl/l (200 ppm) of the isothiazolinone formulation. This type of resistance is stable; in contrary to bacterial persistence there are no disadvantages to organisms possessing the function (Bryan, 1989). Resistance to antimicrobial active agents appears to be a widespread intrinsic characteristic among *Pseudomonas* species (Eagon, 1984). Experience has shown that the problem can be solved (to a limited extent) by application fewer high-dose treatments than more frequent lower dose-treatments, e.g. of metalworking fluids.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

15. HETEROCYCLIC N;S COMPOUNDS

15.4. 2-n-Octyl-4-isothiazolin-3-one (OI)

$\text{C}_{11}\text{H}_{19}\text{NOS}$



Molecular mass

213.34

CAS-No.	26530-20-1
EC-No.	247-761-7
USA Reg.	subject to regulation under FIFRA and therefore exempt from TSCA inventory listing requirements
Synonym/common name	2-n-octyl-3(2H)-isothiazolone
Supplier	ROHM AND HASS; THOR
Chemical and physical properties	
Appearance	clear pale yellow liquid; no distinct odour
Content (%)	~45; solvent: propylene glycol (1.14.)
Boiling point/range °C (101 kPa)	188
Solidification point °C	-40
Density g/ml	1.038
Vapour pressure hPa (25°C) a.i.	4.9×10^{-5} (4.8×10^{-4} at 45°C)
Viscosity mPa·s (20°C)	40
Flash point °C	97
Ignition temperature °C	371 (propylene glycol)
Upper flammability limit % v/v i. air	12.5 (propylene glycol)
Lower flammability limit % v/v i. air	2.6 (propylene glycol)
Log POW (a.i.)	2.45
PH (10% in H ₂ O)	2.4
Stability (a.i.)	thermal stability up to 200°C; in aqueous systems at room temperature stable in the pH range 2–10; sensitive to oxidizing and reducing agents, and to the attack by nucleophiles, e.g. amines, mercaptans
Solubility (a.i.) g/l (25°C)	0.480 in H ₂ O, ~250 in toluene, 5 in hexane; insoluble in heptane; highly soluble in alcohols, acetone, ether, ethyl acetate; partly soluble in oils
Toxicity data (source: ROHM AND HAAS)	
LD ₅₀ oral	760 mg/kg rat
dermal	690 mg/kg rabbit
LC ₅₀ on aerosol inhalation	1.25 mg/l for rats (4 h, nose only)
Skin/eye irritation-tests with rabbits according to OECD Guidelines: corrosive. The product may cause skin sensitization. 2-n-octyl-4-isothiazolin-3-one is neither carcinogenic nor mutagenic and rated "not a teratogen nor reproductive hazard".	
Short-Term Exposure Limit	R. & H. 0.6 mg/m ³
Occupational Exposure Limit	Germany 0.05 mg/m ³ (inhalable aerosol fraction)
Ecotoxicity:	
OI is readily dissipated in river water, activated sludge units, typical soils by biological, chemical and physical means. Toxicity (a.i.) to aquatic species:	
LC ₅₀ for Bluegill sunfish	0.196 mg/l (96 h, static)
Fathead minnow	0.140 mg/l (96 h, static)
Rainbow trout	0.065 mg/l (96 h, static)
LC ₅₀ for Daphnia magna	0.320 mg/l (48 h, static)

Antimicrobial effectiveness/applications

In accordance with its extraordinary high toxicity for fungi the active ingredient is mainly used as a fungicide, e.g. for paint film protection, in non-film forming decorative wood stains, in the leather industry for the protection of wet blues, in adhesives and sealants, pulp, paper and cardboard, etc.. Surface coatings treated with the fungicide may lose mould resistance when exposed to leaching with water because of the relatively high water solubility of OI. Nevertheless the length of a straight chain alkyl group at the N-atom of the isothiazolinone ring has a significant effect on water solubility. The substitution of the methyl group in MI (15.1.) by the n-octyl group causes approx. 100-fold decrease in water solubility. In the search for a non-leachable N-alkyl-isothiazolinone, OI represents an optimum with regard to antimicrobial activity and poor water-solubility.

Table 111 Minimum inhibition concentrations (MIC in mg a.i./l) of 2-n-octyl-4-isothiazolin-3-one (Source: THOR)

Fungi	MIC	Fungi	MIC	Bacteria	MIC
<i>Alternaria alternata</i>	1.5	<i>Phoma</i> sp.	1–2	<i>Corynebacterium</i> sp.	12.5
<i>Aspergillus niger</i>	5–10	<i>Rhizopus stolonifer</i>	4.0	<i>Escherichia coli</i>	62.5
<i>Aspergillus oryzae</i>	2.0	<i>Sclerophoma pithyophila</i>	2.5	<i>Klebsiella</i> sp.	125.0
<i>Aureobasidium pullulans</i>	0.3	<i>Ulocladium atrum</i>	2.5	<i>Proteus</i> sp.	62.5
<i>Chaetomium globosum</i>	4.0		2.0	<i>Pseudomonas aeruginosa</i>	437.5
<i>Cladosporium</i>	0.5	<i>Candida albicans</i> (yeast)	5.0	<i>Pseudomonas stutzeri</i>	100.0
<i>cladosporoides</i>					
<i>Cladosporium resinae</i>	0.5	<i>Rhodotorula rubra</i> (yeast)	1.5	<i>Shewanella</i> sp.	25.0
<i>Fusarium</i> sp.	2.5	<i>Saccharomyces cerevisiae</i> (yeast)	1.0	<i>Staphylococcus aureus</i>	31.5
<i>Gliocladium virens</i>	5.0	<i>Sporobolomyces roseus</i> (yeast)		<i>Streptovorticillium reticulum</i>	10.0
<i>Lentinus tigrinus</i>	2.5			Algae	
<i>Penicillium funiculosum</i>	0.5			<i>Chlorella pyrenoidosa</i>	5.0
<i>Penicillium glaucum</i>	2.5			<i>Nostoc</i> sp.	0.5
<i>Penicillium ochrochloron</i>	0.3			<i>Scenedesmus obliquus</i>	5.0

Microbicide group (substance class)

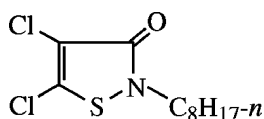
Chemical name

Chemical formula

Structural formula

15. HETEROCYCLIC, N,S COMPOUNDS

15.5. 4,5-Dichloro-2-(n-octyl)-4-isothiazolin-3-one (DCOI)

 $C_{11}H_{17}Cl_2NOS$ 

Molecular mass

282.24

CAS-No.

64359-81-5

EC-No.

264-843-8

EPA Reg.

approval for marine antifoulant application

FIFRA

subject to regulation and therefore exempt from U.S. TSCA Inventory listing requirements

Synonym/common name

4,5-dichloro-2-n-octyl-3(2H)-isothiazolone

Supplier

ROHM AND HAAS; THOR

Chemical and physical properties

Appearance

clear pale yellow liquid; aromatic odour

Content (%)

~30; solvent; xylene

Boiling point °C (101 kPa)

157–174

Solidification point °C, a.i.

–3

Density g/ml (25°C)

0.94 (a.i.: 1.28)

Vapour pressure hPa (16°C)

6.67 (xylene)

Viscosity mPa·s

1.3

Flash point °C

28

Auto ignition temperature °C

530

Upper flammability limit % v/v i. air

7 (xylene)

Lower flammability limit % v/v i. air

1.2 (xylene)

Log POW, a.i.

4.9

Stability

thermal stable (onset of degradation not occurring until 228°C); light stable; stable in the pH range 3–9; sensitive to primary and secondary amines, thiol groups, in addition to strong oxidizing or reducing agents

0.014 in H₂O, ~650 in ethanol, acetone, ~750 in toluene, ~540 in cyclohexane, ~250 in solvent naphtha, ~200 in white spirit

Solubility (a.i.) g/l (25°C)

Toxicity data (source: ROHM AND HAAS)

The following data base on tests conducted with a solution containing 32.6% DCOI in xylene.

LD₅₀ oral

2600 mg/kg female rat

4400 mg/kg male rat

LD₅₀ dermal > 2000 mg/kg rabbit
 LC₅₀ in inhalation for rats 0.26 mg/l a.i./l (4 h aerosol exposure, nose only)

Sensitization-human: Allergic contact dermatitis observed.

Ames (nutagenicity) test: Negative.

Exotoxicity data for the active ingredient:

The half-life of DCOI in aerobic and anaerobic microcosms was less than 1 h; the degradation resulted in products with greatly reduced toxicity. Bioaccumulation studies in fish showed essentially no bioaccumulation of DCOI.

LC ₅₀ for Bluegill sunfish	14 ppb (96 h)
for Rainbow trout	2.7 ppb (96 h)
for Sheepshead minnow	20.5 ppb (96 h)
EC ₅₀ for Daphnia magna	5.2 ppb (48 h)
EC ₅₀ for Mysid shrimp	4.7 ppb (96 h)
EC ₅₀ for algae	20 ppb (96 h)
EC ₅₀ for Bay mussel embryo/larvae	2 ppb (48 h)

Jacobson & Willingham (2000) have compared these data with those found in the literature for tributyltin (TBT) compounds (19.) and state, that TBT in contrary to DCOI shows a wide range of adverse environmental effects.

Antimicrobial effectiveness/applications

The development of DCOI is based on the findings of Miller & Lovegrove (1980) who systematically examined the effect of substitution on the isothiazolinone ring with regard to antimicrobial activity and water solubility. This investigation has shown DCOI to be the preferred candidate, having high biological activity and lowest water solubility. Due to the fact that the molecule contains several toxophoric structural elements – a reactive N-S bond, an activated chloro atom in the α -position to a (electronegative) carbonyl group, a vinyl chloro atom – it is a broad spectrum biostat, toxic for fungi, yeasts, bacteria, algae, sea animals and insects.

DCOI exhibits antimicrobial activity at extremely low inhibitory concentrations (see Table 112). It associates with cells rapidly and extensively, and reacts preferably with intracellular thiols of biocatalysts with the result of enzyme inhibition. In vitro experiments performed by Diehl et al. (1999) demonstrate, that in the sequel of such reactions intermediates appear, which are more inhibitory to SH groups containing enzymes than DCOI itself (see also Part I, Chapter 2).

The very low water solubility of DCOI provides long term activity by resisting leaching. The thermal stability is remarkable, too. Because of these properties DCOI is particularly suitable for the application in antifouling and other surface coatings, in adhesives, sealants, plastics and similar products. Use concentrations are in the range of 0.2–0.6%.

Table 112 Minimum inhibition concentrations (MIC) in mg DCOI/l (Source: THOR)

Bacteria	MIC	Fungi	MIC	Algae	MIC
Bacillus subtilis	2.0	Aspergillus niger	0.07	Cyanobacteria:	
Escherichia coli	18.0	Trichoderma viride	3.0	Anabaena flos-aquae	< 1.0
Pseudomonas aeruginosa	14.0	Ulocladium sp.	0.8	Nostoc commune	< 1.0
Pseudomonas fluorescens	13.0			Microcystis sp.	< 1.0
Staphylococcus aureus	5.0	Candida albicans (yeast)	6.0	Green Algae:	
				Chlorella pyrenoidosa	< 1.0
				Scenedesmus sp.	< 2.0

Microbicide group (substance class)

Chemical name

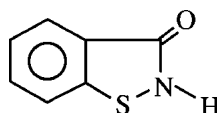
Chemical formula

Structural formula

15. HETEROCYCLIC N,S COMPOUNDS

15.6. 1,2-Benzisothiazolin-3-one (BIT)

C₇H₅NOS



Molecular mass	151.19
CAS-No.	2634-33-5
EC-No.	220-120-9
BgVV (Germany)	recommendation XIV and XXXVI
EPA-Reg.	approval for antimicrobial applications
FDA	various approvals
Synonym/common name	1,2-benzisothiazol-3(2H)-one
Supplier	AVECIA, THOR
Chemical and physical properties	
Appearance	brown liquid
Content (%)	20; alkaline solution in dipropylene glycol and H ₂ O
Boiling point/range °C (101 kPa)	100
Melting point °C of a.i.	approx. 152
Density g/ml (25°C)	1.14
Viscosity mPa·s (25°C)	2.2
Flash point °C	boils without flashing
pH at 25°C	13.5
Stability	thermal decomposition at > 300°C; stable and effective over pH range 4–12; incompatible with some oxidizing and reducing agents e.g. persalts, sulphites etc.;
Solubility	of the a.i.: ~1 g/l H ₂ O; BIT forms water soluble salts with alkalis and amines and is highly soluble in organic solvents, preferably in alcohols and glycols
Toxicity data applying to the a.i. (source: AVECIA)	
LD ₅₀ oral	670 mg/kg male rat 784 mg/kg female rat
LD ₅₀ dermal	> 2000 mg/kg rat
Irritating to skin. May cause sensitization by skin contact. Eye contact can entail serious damage. Studies in animals have shown that repeated doses do not produce teratogenic or foetotoxic effects. In consideration of the structure of BIT and the results of short term tests it is unlikely to be a carcinogenic hazard to man.	
Ecotoxicity:	
LD ₅₀ for Rainbow trout	1.6 mg/l (96 h, flow through)
for Bluegill sunfish	5.9 mg/l (96 h, flow through)
for Brown shrimp	44 mg/l (96 h, semi-static)

Table 113 Minimum inhibition concentrations (MIC) of the 20% BIT formulation (Sources: AVECIA)

Test organisms	MIC (mg/l)
Bacteria	
<i>Bacillus subtilis</i>	40
<i>Enterobacter cloacae</i>	80
<i>Escherichia coli</i>	40
<i>Proteus vulgaris</i>	125
<i>Pseudomonas aeruginosa</i>	250
<i>Pseudomonas putida</i>	250
<i>Staphylococcus aureus</i>	40
<i>Streptococcus faecalis</i>	40
<i>Streptococcus lactis</i>	15
Fungi	
<i>Alternaria alternata</i>	700
<i>Aspergillus niger</i>	350
<i>Aureobasidium pullulans</i>	350
<i>Chaetomium globosum</i>	400
<i>Cladosporium cladosporoides</i>	400
<i>Penicillium notatum</i>	125
Yeasts	
<i>Candida albicans</i>	200
<i>Rhodotorula rubra</i>	400
<i>Saccharomyces cerevisiae (turbidans)</i>	250

EC ₅₀ for <i>Daphnia magna</i>	4.3 mg/l (48 h)
ErC ₅₀ for green algae	0.07 mg/l (r = growth rate)
EbC ₅₀ for green algae	0.15 mg/l (b = biomass)

BIT in a concentration of 5 ppm can be degraded by settled sewage organisms (Bunch and Chambers test).

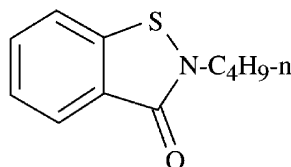
Antimicrobial effectiveness/applications

Preservatives based on BIT may be used for the in-tank/in-can protection of polymer emulsions, latex paints, pigment and filler slurries, paper coatings, adhesives and other aqueous functional fluids. Because of the heat stability and non-volatility of BIT the preservatives can be incorporated in fluids which are still hot. BIT exhibits no headspace activity. It is compatible with non-ionic and anionic compounds and active in acid and alkaline media; the presence of ammonia and amines does not affect the activity of BIT.

Using BIT based preservatives one has to bear in mind that for the control of *Pseudomonads* and some species of fungi (e.g. *Alternaria alternata*) or yeasts (e.g. *Rhodotorula rubra*) one needs higher addition rates than needed for other microbe species. Suggested concentrations on which trials can be based are in the range 0.05–0.25%.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

15. HETEROCYCLIC N,S COMPOUNDS
15.7. N-Butyl-1,2-benzisothiazolin-3-one (BBIT)
C₁₁H₁₃NOS



Molecular mass 207
CAS-No. 4299-07-04
ELINCS-No. 420-590-7 (EC-List of Notified Chemical Substances)
Synonym/common name N-butyl-1,2-benzisothiazol-3(2H)-one
Supplier AVECIA

Chemical and physical properties

Appearance brown, almost odourless oil
Content (%) ~100
Boiling point/range °C (101 kPa) decomposes before boiling
Density g/ml (20°C) approx. 1.17
Vapour pressure hPa (25°C) 0.00015
Flash point °C approx. 178
Auto ignition temperature °C approx. 425
Log POW 2.86
Stability thermal decomposition at approx. 300°C; stable over a wide pH range (2–12); reacts with thiols
Solubility g/l < 0.0005 in H₂O; highly soluble in ethanol and most other organic solvents

Toxicity data (Source: AVECIA)

LD₅₀ oral > 2000 mg/kg rat
dermal > 2000 mg/kg rat

Causes burns on the skin and is corrosive to the eyes and mucous membranes. May cause sensitization by skin contact.

Ecotoxicity: BBIT is essentially insoluble in water, has a low potential for bioaccumulation and is classified as not readily biodegradable.

LC ₅₀ for Rainbow trout	0.15 mg/l (96 h, flow through)
EC ₅₀ for <i>Daphnia magna</i>	0.093 mg/l (48 h)
EbC ₅₀ for green algae	0.24 mg/l (72 h; b = biomass)

ErC ₅₀ for green algae	0.45 mg/l (72 h; r = growth rate)
IC ₁₀ for <i>Pseudomonas putida</i>	~0.2 mg/l (6 h; growth rate)

Antimicrobial effectiveness/application

The MIC's listed in Table 114. demonstrate the broad spectrum of effectiveness of BBIT which covers fungi, yeasts, algae and bacteria. However, the user should mind that the inhibition/inactivation of *Pseudomonads*, requires BBIT concentrations which exceed considerably those necessary for the control of other microbe species. BBIT is effective between pH 2 and 12. Its liquid nature and its high degree of chemical and thermal stability favour its application for the protection of plastics or metalworking fluids against biodeterioration. For trial purposes diluted metalworking fluids should contain 0.005–0.02% of BBIT. The incorporation into plastics is facilitated by the possibility to dissolve BBIT in plasticisers.

Table 114 Minimum inhibition concentrations (MIC) of N-butyl-1,2-benzisothiazolin-3-one (Source: AVECIA)

Test organism	MIC (mg/l)
<i>Altenaria alternata</i>	25
<i>Aspergillus niger</i>	16
<i>Aureobasidium pullulans</i>	4
<i>Chaetomium globosum</i>	1.6
<i>Cladosporium cladosporioides</i>	19
<i>Cladosporium herbarum</i>	2.3
<i>Gleocladium virens</i>	62
<i>Phoma violacea</i>	2
<i>Paecilomyces varioti</i>	4
<i>Penicillium funiculosum</i>	3
<i>Scopulariopsis brevicaulis</i>	37
<i>Trichoderma viride</i>	50
<i>Acinetobacter baumannii</i>	14
<i>Alcaligenes faecalis</i>	20
<i>Bacillus subtilis</i>	4
<i>Burkholderia cepacia</i>	31
<i>Corynebacterium sp.</i>	1.4
<i>Escherichia coli</i>	87.5
<i>Flavobacterium capsulatum</i>	11
<i>Klebsiella pneumoniae</i>	137
<i>Lactobacillus brevis</i>	2.1
<i>Proteus mirabilis</i>	137
<i>Pseudomonas aeruginosa</i>	200
<i>Pseudomonas fluorescens</i>	100
<i>Pseudomonas putida</i>	50
<i>Staphylococcus aureus</i>	2.5
<i>Streptoverticillium waksmanii</i>	2
<i>Oscillatoria tenuis</i>	16
<i>Trentopholia aurea</i>	16
<i>Chlorella vulgaris</i>	16
<i>Nostoc muscarum</i>	16
<i>Pleurococcus sp.</i>	16
<i>Candida albicans</i>	19
<i>Rhodotorula mucilaginosa</i>	4
<i>Saccharomyces cerevisiae</i>	0.4

Microbicide group (substance class)

Chemical name

Chemical formula

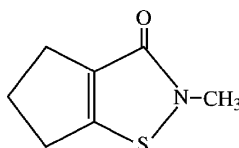
Structural formula

Molecular mass

15. HETEROCYCLIC N, S COMPOUNDS

15.8. 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one
(MTI)

C₇ H₉ NOS



155.22

CAS-No.	82633-79-2
EC-No.	407-630-9
Synonym/common name	5,6-dihydro-2-methyl-2H-cyclopent[d]iso-thiazol-3-(4H)-one
Supplier	AVECIA
Chemical and physical properties	
Appearance	mobile, pale straw coloured, odourless liquid
Content (%)	5 (aqueous solution)
Boiling point/range °C (101 kPa)	approx. 100
Solidification point °C	-2
Density g/ml (25°C)	1.02
Vapour pressure hPa (25°C)	0.0017
Viscosity mPa·s (25°C)	approx. 1.1
Flash point °C	boils without flashing
Log POW of MTI (25°C)	0.6
pH	approx. 4–6
Stability	the solution is stable under normal storage conditions; the a.i. starts to decompose at 216°C; it is relatively stable in an aqueous solution, whose pH had been adjusted to 9 by ammonia (Eacott, 1991), that means significantly more stable than 5-chloro-2-methyl-4-isothiazolin-3-one (15.2.), but less stable than 1,2-benzisothiazolin-3-one (15.6.)
Solubility	may be diluted with H ₂ O
Toxicity data of MTI (source: AVECIA)	
LD ₅₀ oral	168–224 mg/kg rat
dermal	> 1000 mg/kg rat
Short term tests have shown that MTI is unlikely to be a carcinogenic hazard to man. MTI is irritant to skin and mucous membranes and can cause severe eye irritation. It is a potent skin sensitizer; however, according to Botham et al. (1990) the sensitizing potency is lower than that of 5-chloro-2-methyl-4-isothiazolin-3-one (15.2).	
Ecotoxicity: Tests show that MTI is completely biodegradable at concentrations below the biocidal level. It inhibits the respiration rate of activated sludge with an IC ₅₀ of 18 mg/l and a no effect concentration of 3.2 mg/l.	
LC ₅₀ for Rainbow trout	0.97 mg/l (96 h, semi-static)
EC ₅₀ for Daphnia	1.2 mg/l (48 h)
EbC ₅₀ for green algae	0.28 mg/l (96 h; b = biomass)
ErC ₅₀ for green algae	0.55 mg/l (96 h; r = growth rate)

Table 115 Minimum inhibition concentrations (MIC) of MTI in nutrient agar (Source: AVECIA)

Test organisms	MIC (mg/l)
Bacteria	
<i>Pseudomonas aeruginosa</i>	20
<i>Aeromonas hydrophila</i>	5
<i>Klebsiella pneumoniae</i>	10
<i>Serratia marcescens</i>	10
<i>Proteus mirabilis</i>	40
<i>Proteus rettgeri</i>	40
<i>Enterococcus faecalis</i>	5
Fungi	
<i>Candida lipolytica</i> (Yeast)	10
<i>Candida boidinii</i> (Yeast)	5
<i>Pichia bispora</i> (Yeast)	5
<i>Geotrichum candidum</i>	40
<i>Alternaria alternata</i>	40
<i>Aspergillus niger</i>	40
<i>Penicillium funiculosum</i>	20

Antimicrobial effectiveness/applications

The proliferation of bacteria, fungi and yeasts is inhibited by MTI concentrations (MIC) listed in Table 115.

The low partition coefficient (log POW) favours the application of MTI for the in-can/in-tank protection of two-phase systems, such as aqueous paints and polymer emulsions. At pH values of 6 to 9 the efficacy of MTI is not adversely affected. Tests have clearly shown that MTI exhibits its microbistatic/microbicidal efficacy in contaminated products comparatively slowly (it may extend over several days). Suggested addition rates move between 50 and 100 ppm (0.1–0.2% of the 5% preparation).

Microbicide group (substance class)

Chemical name

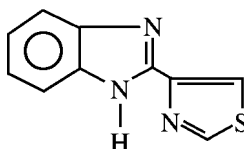
Chemical formula

Structural formula

15. HETEROCYCLIC N, S COMPOUNDS

15.9. 2-(1,3-Thiazol-4-yl) benzimidazole (TBZ)

C₁₀ H₇ N₃S



Molecular mass

CAS-No.

EC-No.

EPA FIFRA 1988

EPA TSCA

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Density g/ml (20°C)

Stability

Solubility g/l

201.25

148-79-8

205-725-8; food additive E 233

Pesticide subject to registration or re-registration

Section 8 (B) Chemical Inventory

4-(2-benzimidazolyl)thiazole, Thiabendazole

MERCK, SIGMA-ALDRICH

off white, free flowing powder

~100

304–305 (sublimes)

1.44

heat resistant; stable in acid and alkaline media

0.03 in H₂O, 38.4 in water of pH 2.2, 2.8 in acetone, 2.3 in benzene, 39.0 in DMF, 80.0 in DMSO, 4.5 in dioxane, 6.8 in ethanol, 7.7 in ethylene glycol, 9.3 in methanol

Toxicity data

LD₅₀ oral

2080 mg/kg rat

1300 mg/kg mouse

3850 mg/kg rabbit

Skin compatible.

Table 116 Minimum inhibition concentrations (MIC) of TBZ in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	> 5000
<i>Aspergillus niger</i>	40
<i>Aureobasidium pullulans</i>	1
<i>Chaetomium globosum</i>	1
<i>Cladosporium herbarum</i>	< 1
<i>Coniophora puteana</i>	100
<i>Diplodia natalensis</i>	3
<i>Lentinus tigrinus</i>	> 5000
<i>Penicillium digitatum</i>	50
<i>Penicillium glaucum</i>	< 1
<i>Penicillium italicum</i>	50
<i>Polyporus versicolor</i>	20
<i>Trichoderma viride</i>	10
<i>Candida albicans</i>	> 1000
<i>Candida krusei</i>	> 1000
<i>Rhodotorula mucilaginosa</i>	35
<i>Sporobolomyces roseus</i>	75
<i>Torula rubra</i>	150

Antimicrobial effectiveness/application

TBZ is primarily fungistatic and of special interest because of its effectiveness against *Penicillium digitatum*, *Penicillium italicum*, *Diplodia natalensis* and *Gloeosporium* species which led to its use for the protection of citrus fruits and bananas. It has also proposed to use TBZ as a fungicide in paint compositions, in adhesives, sealants, paper (e.g. soap wrappers), cardboard, fibreboard fabric, leather goods, etc., where one can profit from the advantages of TBZ: poor water-solubility, non-volatility, stability, heat resistance and low toxicity.

However, there are deep gaps in the activity spectrum of TBZ (see Table 116) similar to those of Carbendazim (11.4.). It is therefore recommendable to combine TBZ with other fungicides, e.g. Ziram (11.11.3.), Zinc Pyrithione (13.1.3b.), Dichlofluanide (16.5.), Tolyfluanide (16.6.) and others. Specific mixtures were proposed by Brake (1974). In a research on fungicides for aerial disinfection by thermal fogging in libraries and archives Rakotonirainy et al. (1999) found TBZ effective and most suitable when a 10% preparation was aerosolized at a rate of 5 ml/m³.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Density g/ml (20°C)

Stability

Solubility

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

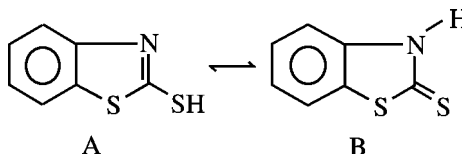
Chemical and physical properties of an aqueous solution

Appearance

Content (%)

15. HETEROCYCLIC N, S COMPOUNDS

15.10. 2-Mercaptobenzothiazole (A) ↔ Benzothiazolin-2-thione(B)

C₇ H₅ NS₂

167.26

149-30-4

205-736-8

Data base, Jan. 2001

MBT, 2-benzothiazolyl mercaptan, 2(3H)-benzothiazolethione
SIGMA-ALDRICHslightly yellow, crystalline powder with an unpleasant odour
~100

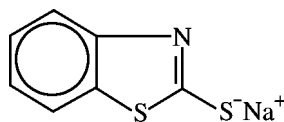
182

1.42–1.52

heat resistant; stable in alkaline solutions (formation of corresponding salts)

sparingly soluble in H₂O and acids; soluble in alkaline solutions; highly soluble in acetone, moderately soluble in alcohol, ether, benzene

15.10a. 2-Mercaptobenzothiazole sodium salt (MBT-Na)

C₇ H₄ NNaS₂

189.25

2492-26-4

219-660-8

sodium benzothiazol-2-yl sulphide

yellow-brown fluid

50

Boiling point/range °C (101 kPa)	~108
Solidification point °C	~-14
Density g/ml (20°C)	~1.26
Vapour pressure hPa (20°C)	~20
Viscosity mPa·s (20°C)	< 90
Flash point °C	> 108
Ignition temperature °C	~480
pH (1% in H ₂ O)	~9.5
Stability	stable between pH 7 and 14
Solubility	miscible with water, alkalis, ethylene glycol, propylene glycol

Toxicity data of MBT (15.10.)

LD ₅₀ oral	1158 mg/kg mouse
intraperitoneal	100 mg/kg mouse
	300 mg/kg rat
LD ₅₀ dermal	> 7940 mg/kg rabbit
LC ₅₀ on inhalation	> 1270 mg/m ³ for rats

Carcinogenic by RTECS criteria.

MBT may cause sensitization on skin contact. The MBT-Na solution is severely irritant to skin, eyes and mucous membranes.

Toxicity to fish

LC₅₀ for *Leuciscus idus* 5 mg MBT-Na (50%)/l (48 h)

Antimicrobial effectiveness/applications

As can be seen from the structural formula for the tautomeric form B of MBT, the dithiocarbamate configuration $\begin{smallmatrix} \text{N}-\text{C}-\text{S}- \\ | \quad | \\ \text{H} \quad \text{S} \end{smallmatrix}$ is a structural element of the MBT molecule. Its antimicrobial efficacy is due to its chelation qualities (Albert et al., 1947). The minimum inhibition concentrations in Table 47 inform about the activity spectrum of MBT in comparison to the corresponding N-hydroxymethyl derivative (3.4.12.) which is a formaldehyde releasing substance. As a fungicide MBT is especially toxic to superficial moulds and cellulose-decomposing fungi. The antibacterial activity of MBT is marked by a lack of efficacy for Pseudomonads. In practical application, mainly as slimicides, MBT sodium salt solutions therefore contain additionally other dithiocarbamates (e.g. 11.10.1., 11.11.1.). Moreover MBT/sodium MBT is characterized by dual utility, as it serves also as a popular corrosion inhibitor for non-ferrous metals.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA Reg.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

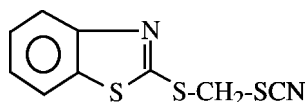
Density g/ml (22°C)

Vapour pressure hPa (25°C)

15. HETEROCYCLIC N, S COMPOUNDS

15.11. 2-(Thiocyanomethylthio)benzthiazole (TCMBT)

C₉ H₆ N₂ S₃



238.36

21564-17-0

244-445-0

approval for antimicrobial applications

(benzothiazol-2-ylthio)methyl thiocyanate

BAYER, BUCKMAN, THOR

yellow crystals or oil

> 98

1.38

4.75×10^{-6}

Stability	extremely reactive/incompatible with alkalis; cyanide salts are formed in contact with strong alkali; thermal decomposition in an 30% emulsifiable acidic formulation: T 1/2 70°C: 3.26 years; T 1/2 120°C: 92 hours
Solubility g/l	0.03 in H ₂ O, <0.5 in isopropanol, ethylene glycol, propylene glycol; highly soluble in acetone, DMF, ethyl glycolacetate, toluene

Toxicity data (source: THOR)

LD ₅₀ oral	500 mg/kg rat
dermal	1414 mg/kg rat

Toxic by inhalation of sprays or aerosols. Irritant to skin and mucous membranes; strongly irritant (corrosive) to the eyes. Sensitization possible by skin contact.

Ecotoxicity (source: BUCKMAN):

Toxic to aquatic organisms, LC₅₀ for trouts: 0.1 mg/l (96 h).

Biodegradable (Bunch-Chambers).

Antimicrobial effectiveness/application

TCMBT is available in formulations containing 80%, 60%, 30%, or 20% active ingredient. As is demonstrated by the MIC's in Table.116 it is a broad spectrum microbistat, active against fungi, bacteria and algae. However the centre of TCMTB's efficacy is directed against fungi. Important application fields for TCMBT: Leather industry (protection of pickled pelts and wet blues during stock); wood and particle board protection (especially temporary protection of sawn timber against blue stain and fungal infestation); application in slimicides and preservatives for the paper industry, as a microbicide for the inhibition of biofouling in cooling water.

Table 117 Minimum inhibition concentrations (MIC in mg/l) of a 60% TCMBT formulation for various micro-organisms in nutrient agar (Source: BAYER)

Test Organism	MIC
<i>Alternaria tenuis</i>	5
<i>Aspergillus niger</i>	5
<i>Aureobasidium pullulans</i>	15
<i>Chaetomium globosum</i>	2
<i>Lentinus tigrinus</i>	2
<i>Penicillium glaucum</i>	5
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	35
<i>Escherichia coli</i>	20
<i>Pseudomonas aeruginosa</i>	50
<i>Staphylococcus aureus</i>	35
Algae	10-20

16. *N*-Haloalkylthio compounds

N-Haloalkylthio compounds with antimicrobial activity are obtained in almost any desired number according to the following reaction scheme, provided that the H atom has acidic character:



The most important microbicides within this substance class are the compounds bearing a trihalomethylthio group (S-CX₃) as toxophor. The antimicrobial efficacy of *N*-haloalkylthio compounds is based on the capacity of the N-S bond to open and react with nucleophilic components of the microbial cell (Paulus & Kühle, 1986). The most effective compounds are those containing the S-CCl₂F group. These also contrary to the trichloromethylthio derivatives do not show mutagenic activities (Schuphan et al., 1981). Optimum antimicrobial activity is achieved with compounds whose N-S bond has a medium stability.* Taking account of the facts mentioned, it is possible to select from the wide range of *N*-haloalkylthio compounds already available, microbicides with such chemico-physical and toxicological properties as are suited to defined uses, e.g. as fungicides and algicides in plastics, paint films, wood preservatives and antifouling coatings.

* See Part I, Chapter 2.

Table 118 Minimum inhibition concentrations (MIC) (mg/litre) of *N*-trihalomethylthio microbicides – comparison of *N*-S-CCl₂F compounds with *N*-S-CCl₃ compounds

Test organism	Dichlofluanide, <i>N,N</i> -dimethyl- <i>N'</i> -phenyl- <i>N'</i> -fluorodichloromethylthio-sulphamide	<i>N,N</i> -dimethyl- <i>N'</i> -phenyl- <i>N'</i> -trichloro-methylthio-sulphanide	Fluorofolpet, <i>N</i> -fluorodichloromethylthio-phthalimide	Folpet, <i>N'</i> -trichloromethylthio-phthalimide
<i>Alternaria alternata</i>	10	1000	10	750
<i>Aspergillus niger</i>	50	> 4000	200	350
<i>Aureobasidium pullulans</i>	10	200	50	50
<i>Chaetomium globosum</i>	20	1000	100	50
<i>Coniophora puteana</i>	10	500	2	75
<i>Lentinus tigrinus</i>	2	200	2	35
<i>Penicillium glaucum</i>	20	4000	35	50
<i>Polyporus versicolor</i>	10	> 1000	50	100
<i>Sclerophoma pityophila</i>	10	500	10	150
<i>Trichoderma viride</i>	≥5000	> 5000	1000	> 5000
<i>Trichiophyton pedis</i>	10		20	
<i>Candida albicans</i>	20	> 5000	5	150
<i>Candida krusei</i>	20	> 5000	20	500
<i>Rhodotorula mucilaginosa</i>	20	> 5000	10	100
<i>Saccharomyces cerevisiae</i>	10	750	5	50
<i>Torula rubra</i>	20	> 5000	15	50
<i>Torula utilis</i>	10	350	10	50
Algae	10	50	20	20

In Table 118 the minimum inhibition concentrations (MIC) of two *N*-S-CCl₂F compounds (Dichlofluanide and Fluorofolpet) are compared with the MIC of the corresponding *N*-S-CCl₃ compounds. The higher efficacy of the *N*-S-CCl₂F compounds is demonstrated. Remarkable is the resistance of *Trichoderma viride* against the *N*-trihalomethylthio compounds listed in Table 118.

Microbicide group (substance class)

Chemical name

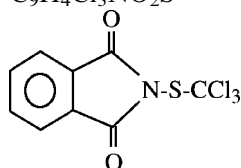
Chemical formula

Structural formula

16. N-HALOALKYLTHIO COMPOUNDS

16.1. *N*-(Trichloromethylthio)phthalimide

C₉H₄Cl₃NO₂S



Molecular mass

296.57

CAS-No.

133-07-3

EC-No.

205-088-6

EPA Reg.

for antimicrobial application

EPA TSCA

Section 8 (B) Chemical Inventory – on EPA IRIS Data base

Synonym/common name

2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione,
Folpet, Phaltan

Supplier

INTERNATIONAL SPECIALTY PRODUCTS (ISP),
SIGMA-ALDRICH

Chemical and physical properties

Appearance

off-white crystalline powder with a faint mercaptaneous odour

Content (%)

min. 88

Melting point °C

177

Bulk density g/l (20°C)

1600

Vapour pressure hPa (20°C)

< 1.73 × 10⁻⁵

Ignition temperature °C

> 400

Stability

thermal decomposition starts at 180°C; hydrolyzes in aqueous media increasingly with increase of pH and temperature (in solution at pH 7.8 100% decomposition at 20°C within 2 h); reacts under decomposition with sulphides and thiol compounds, e.g. cysteine (see Part I, Chapter 2, Figure 19)

Solubility g/l	< 1 in H ₂ O, 30–40 in ketones, 1–10 in non-polar solvents
Toxicity data	
LD ₅₀ oral	2636 mg/kg rat 1546 mg/kg mouse
LD ₅₀ intraperitoneal	68.4 mg/kg rat 80 mg/kg mouse
LC ₅₀ on inhalation	> 5 g/m ³ (2 h) for rats > 6 g/m ³ (2 h) for mice
LD ₅₀ dermal	> 22.6 g/kg rabbit

Only moderately irritant to the skin, however, of irritating effect to mucous membranes and eyes. Sensitization possible through skin contact.

Rated as probably carcinogenic by EPA classification; tumorigenic agent by RTECS criteria. See also reports of McCann & Ames, 1975, Moriya et al., 1983 and Gold et al., 1984. Ecotoxicity (Paulus, 1993):

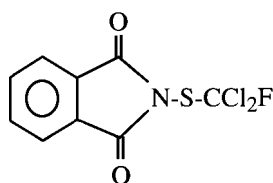
Hydrolyses in water to H₂S, HCl, CO₂ and phthalimide. LC₀ of phthalimide for fish: approx. 800 mg/litre. Activated sludge organisms tolerate up to 1000 mg phthalimide/litre without losing activity.

Antimicrobial effectiveness/applications

Folpet's antimicrobial activity is particularly directed against fungi (Table 118). It was developed by CHEVRON (1949) as a fungicide for plant protection. The application of Folpet as a fungicide for the protection of materials is limited by its poor solubility properties; another limitation is its tendency to hydrolyse in aqueous media. Therefore Folpet is usually applied as a fungicide in solvent-based pigmented coatings or wood stains with a relatively high resin content. It is also designed for use in various plastic and vinyl compositions if the processing temperature does not exceed 180°C. The excellent performance of Folpet in these substrates is underlined by its efficacy against "pink-staining" micro-organisms.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

16. N-HALOALKYLTHIO COMPOUNDS
16.2. N-(Fluorodichloromethylthio)phthalimide
C₉H₄Cl₂FNO₂S



Molecular mass
CAS-No.
EC-No.
Synonym/common name
Supplier

280.11
719-96-0
211-952-3
Fluorfolpet, Fluorphaltan
BAYER

Chemical and physical properties

Appearance
Content (%)
Melting point °C
Density g/ml (20°C)
Vapour pressure hPa (20°C)
Ignition temperature °C
Stability

white, crystalline powder with a faint musty odour
min. 96
~145
approx. 1.89
3.65 × 10⁻⁶; (6.37 × 10⁻⁵ at 50°C, < 10⁻¹ at 100°C)
approx. 440
slight thermal decomposition begins at approx. 150°C, noticeable decomposition at 200°C; hydrolyzes in aqueous solutions (100% decomposition at pH 7.8 within 0.3 h at 20°C); incompatible with alkalis, sulphides and R-SH compounds
0.015 in H₂O, ~20 in dioctyl phthalate, ~40 in dibenzyl phthalate, ~20–40 in alkyd resins, ~120 in acetone

Solubility g/l (20°C)

Toxicity data (source: BAYER)

LD ₅₀ oral	2900 mg/kg rat ~2500 mg/kg cat ~1000 mg/kg rabbit
LD ₅₀ dermal	> 1000 mg/kg rat (4 h); the dose of 1000 mg/kg caused no symptoms.

Subacute toxicity: In an animal test the lowest doses tested (20 mg/kg/day) only resulted in slight toxic effects following repeated oral administration over a period of 28 days. In tests with rabbits (exposure 24 h) Fluorfolpet caused moderate skin and eye irritation. It acted as a skin sensitizer in the guinea pig test.

Ecotoxicity:

LD ₀ for fish (<i>Leuciscus idus</i>)	2 mg/l (48 h)
EC ₅₀ for the respiration inhibition of activated sludge	~4.6 mg/l (OECD 209)

The product is not easily biodegradable. It hydrolyzes in water under generation of phthalimide which disposes of only low fish and bacterial toxicity:

LC ₀ for <i>Leuciscus idus</i>	800 mg/l (48 h)
EC ₀ for activated sludge organisms	~1000 mg/l

Antimicrobial effectiveness/applications

The spectrum of activity of Fluorfolpet (see Table 118) covers all types of mould fungus, including *Streptomyces reticulum* (causes pink stain), which may infest surfaces of plastic material and paint films. Remarkable is also the algicidal effect of Fluorfolpet and its potential to inhibit sessile marine organisms like *Balanus* species and tube worms. As Fluorfolpet is virtually insoluble in water, non-volatile, light- and thermo-stable it is used for the formulation of non-aqueous mould-resistant and algicidal coatings for outdoor use and for the fungicidal treatment of plastics, in particular plasticised PVC, and in marine antifouling coatings.

According to a report of Upsher & Roseblade (1984) plastic material containing Fluorfolpet performed very well even under extreme severe exposure conditions (tropical climate) for 3 years.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCA

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

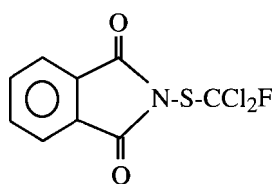
Melting point °C

Density g/ml (20°C)

pH (1% suspension i. H₂O)

16. N-HALOALKYLTHIO COMPOUNDS

16.3. N-(Trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide

C₉H₈Cl₃NO₂S

300.61

133-06-2

205-087-0

Section 8 (B) Chemical Inventory

Data Base, Jan. 2001

N-(trichloromethylthio)tetrahydrophthalimide, 3a, 4,7,7a-tetrahydro-2-(trichloromethane-sulphenyl)-1,3-isoindoldione, Captan

ISP/CREANOVA, SIGMA-ALDRICH

white to buff coloured amorphous powder with a characteristic odour

97

171–175

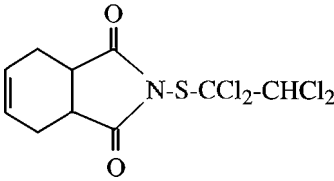
1.7

5–6

Stability	heat resistant under PVC processing temperatures; most stable in non-aqueous, non alkaline systems; hydrolyzes rapidly in aqueous media depending on pH and temperature, being slower at pH 4 and rapid at pH above 10 at a constant temperature
Solubility	virtually insoluble in H ₂ O (< 2 mg/l), soluble in acetone, ethanol, kerosene, xylene, chloroform and benzene
Toxicity data (selection)	
LD ₅₀ oral	9000 mg/kg rat 7000 mg/kg mouse
LD ₅₀ dermal	> 5000 mg/kg rat
LD ₅₀ intraperitoneal	30 mg/kg mouse
LC ₅₀ on inhalation	> 5700 mg/m ³ (2 h) for rats 4500 mg/m ³ (2 h) for mice
Only moderately irritant to skin, irritant to eyes and mucosa; disposes of sensitization potential.	
Mutagenic (Schuphan et al., 1981; Xu & Schurr, 1990).	
Carcinogenic (Heil et al., 1991).	
Rated as possibly carcinogenic by EPA classification.	
Occupational exposure limit 5 mg/m ³	

Antimicrobial effectiveness/applications

The activity spectrum of Captan covers Gram-positive and Gram-negative bacteria, fungi and yeasts; the minimum inhibition concentrations are in the range of 25–100 mg Captan/ml nutrient agar. Captan has been recommended for the microbicidal treatment of non-aqueous coatings and plastic materials, especially PVC mixtures. The addition rates move between 0.5% and 2% based on total paint weight. For use in general vinyl applications such as wall coverings, seat covers, shower curtains, awnings and similar applications use levels of 0.25% to 0.75% (depending on the susceptibility of the systems) based on plasticizer weight are recommended.

Microbicide group (substance class)	16. N-HALOALKYLTHIO COMPOUNDS
Chemical name	16.4. N-1,1,2,2-Tetrachloroethylthio-tetrahydrophthalimide
Chemical formula	C ₁₀ H ₉ Cl ₄ NO ₂ S
Structural formula	
Molecular mass	349.07
CAS-No.	2425-06-1
EC-No.	219-363-3
EPA/NTP	Classification: carcinogenic
Synonym/common name	N-1,1,2,2,-tetrachloroethylthio-4-cyclohexene-1,2-dicarboximide, Captafol
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	yellowish crystalline powder
Content (%)	~100
Melting point °C	159–161
Flash point °C	> 100
Stability	hydrolytic degradation in aqueous media, rapidly in alkaline media
Solubility	sparingly soluble in H ₂ O, soluble in organic solvents

Toxicity data

LD₅₀ oral 2500 mg/kg ratLD₅₀ dermal 15.4 g/kg rabbit

Carcinogenic according to RTECS criteria. – Teratogenic.

Irritant to skin and mucosa. May cause sensitization.

Occupational exposure limits (CH, DK, UK) 0.1 mg/m³

Ecotoxicity: Toxic for aquatic organisms.

LC₅₀ for fish: *Carassius auratus* 0.15–0.4 mg/l (96 h)*Onchorhynchus mykiss* 0.02–0.06 mg/l (96 h)

others 0.03 mg/l (96 h)

EC₅₀ for *Daphnia magna* < 0.001 mg/l (48 h)*Antimicrobial effectiveness/applications*

Captafol exhibits significant antifungal activity. It has been developed by CHEVRON 1965 as an agricultural fungicide. Attempts to use Captafol as a fungicide for the protection of materials, in particular wood, were not very successful.

Microbicide group (substance class)

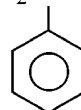
Chemical name

Chemical formula

Structural formula

16. N-HALOALKYLTHIO COMPOUNDS

16.5. N-N-Dimethyl-N'-phenyl-N'-dichlorofluoromethylthiosulphamide

C₉H₁₁Cl₂FN₂O₂S₂(CH₃)₂N-SO₂-N-S-CCl₂F

Molecular mass

333.24

CAS-No.

1085-98-9

EC-No.

214-118-7

Japan

MITI Reg.

Switzerland

BAG.T-no. 17294

Australia

AICS listed

EPA TSCATS

Data Base, Jan. 2001

Synonym/common name

N-dimethylaminosulphonyl-N-phenyl-dichlorofluoromethanesulphenamide, N-(dichlorofluoromethylthio)-N-(dimethylsulphamoyl)-aniline, Dichlofluamide
BAYER

Supplier

Chemical and physical properties

Appearance

white to pale yellow powder with a faint musty odour

Content (%)

~93 (contains approx. 6% insoluble inert material)

Melting point °C

approx. 105

Bulk density g/l (20°C)

approx. 400

Vapour pressure hPa (20°C)

2.15 × 10⁻⁷ (at 50°C: 3.03 × 10⁻⁵)

Flash point °C

approx. 192

Ignition temperature °C

approx. 290

Exothermic reaction

starts at 206°C

Log POW

3.7

Stability

hydrolyzes in aqueous media, is incompatible with alkalis and amines, sulphides and thiol compounds; hydrolysis leads to the generation of N,N-dimethyl-N'-phenylsulphamide (DMSA)

Solubility g/l (20°C)

pure a.i.: 0.0013 in H₂O, 300 in acetone, 260 in cyclohexanone, 130 in ethyl glycol acetate, 117 in ethyl acetate, 75 in Solvesso 100, 65 in xylene, 61 in isooctyl alcohol, 20 in linseed oil, 12 in white spirit

Toxicity data (source: BAYER)

LD ₅₀ oral	> 5000 mg/kg rat
LD ₅₀ dermal	> 2000 mg/kg rabbit (24 h)
	> 5000 mg/kg rat (24 h); the dosage of 5000 mg/kg caused no symptoms
LC ₅₀ on inhalation	approx. 1.3 mg dust/l air (4 h) for rats

In tests with rabbits moderately irritant to skin and eyes. In the guinea pig test the product has a sensitizing effect.

In the literature (e.g. Moriya et al., 1983; Barrueco & de la Pena, 1988; Heil et al., 1991) one finds controversial results with regard to investigations on mutagenic or carcinogenic effects.

Ecotoxicity: According to the closed bottle test (BOD-determination) Dichlofluanide is rated as moderately biodegradable (20 to 30%).

Abiotic degradation: half time (pure a.i.) at 22°C

t 1/2 > 15 d at pH 4 (22°C)

t 1/2 > 18 h at pH 7 (22°C)

t 1/2 ≤ 10 min at pH 9 (22°C)

LC ₅₀ for fish (Rainbow trout)	0.05 mg/l (96 h)
EC ₅₀ for <i>Daphnia magna</i>	0.42 mg/l (48 h)
Inhibition of waste water bacteria: IC ₅₀	19 mg/l

The hydrolysis product DMSA is significantly less ecotoxic than intact Dichlofluanide:

LC ₀ for <i>Leuciscus idus</i>	125 mg/l (48 h)

Waste water bacteria tolerate approx. 200 mg DMSA/l without losing activity.

Antimicrobial effectiveness/applications

Dichlofluanide has a broad spectrum of activity (see Table 118) which covers fungi, including mercury resistant *Penicillium* species, and yeasts. It is particularly effective against wood-staining fungi (blue stain mould) and therefore one of the most important fungicides in solvent-based non-film forming decorative wood stains, in wood coatings and primers. For extended wood protection which includes protection against wood destroying fungi, combinations of Dichlofluanide with corresponding fungicides such as Tebuconazole (14.1.) have shown excellent performance. In such applications Dichlofluanide may be combined additionally with an insecticide, e.g. Cyfluthrin.

Favourable toxicity data, insolubility in water, non-volatility, light stability and excellent algicidal effectiveness are advantages of Dichlofluanide which favour its use in solvent-based surface coatings including antifouling coatings. In marine paints Dichlofluanide is considered as a non-ecotoxic alternative to organo-tin compounds (19.) which are under pressure for substitution. As Dichlofluanide is not phytotoxic – it was developed by BAYER 1964 as a plant protection fungicide – coatings and sealants containing the microbicide can safely be used in green houses –.

Suggested addition rates:

0.5 – 0.7% in wood stains

1.5 – 2.5% in solvent based coatings, calculated on film weight

2.5 – 5% in marine antifouling paints, calculated on total formulation

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

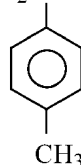
Molecular mass

16. N-HALOALKYLTHIO COMPOUNDS

16.6. N,N-dimethyl-N'-tolyl-N'-dichlorofluoromethylthio-sulphamide

C₁₀H₁₃Cl₂FN₂O₂S₂

(CH₃)₂N-SO₂-N-S-CCl₂F



347.26

CAS-No.	731-27-1
EC-No.	211-986-9
Australia	ACICS listed
Canada	CEPA/DSL listed
Switzerland	BAG, TNo. 54191
Synonym/common name	N-dimethylaminosulphonyl-N-tolyl-dichlorofluoromethanesulphenamide, N-(dichlorofluoromethylthio)-N-(dimethylsulphamoyl)-p-toluidine, Tolyfluanide
Supplier	BAYER
Chemical and physical properties	
Appearance	white powder with a weak odour
Content (%)	min 96
Melting point °C	approx. 95
Density g/ml (20°C)	approx. 1.5
Vapour pressure hPa (20°C)	pure a.i.: 2×10^{-6} (at 50°C: 1×10^{-4})
Flash point	approx. 192
Ignition temperature °C	approx. 400
Exothermic reaction	starts at 204°C
Log POW	3.9
Stability	hydrolyses in aqueous media, is incompatible with alkalis and amines, sulphides and thiol compounds
Solubility g a.i./l (20°C)	approx. 0.002 i. H ₂ O, ~300 in ethyl acetate, ~220 in Xylene, ~120 in Shellsol AB, ~180 in Solvesso 100, ~15 in white spirit

Toxicity data (source: BAYER)

LD ₅₀ oral	> 5000 mg/kg rat
LD ₅₀ dermal	> 5000 mg/kg rat
LC ₅₀ on inhalation	> 1038 mg as aerosol/m ³ (4 h) for rats

In tests with rabbits irritant to the skin, severe eye irritation. The product is irritating to the respiratory system. It has a sensitizing effect in the guinea pig test.

Tolyfluanide is neither carcinogenic nor mutagenic nor teratogenic.

Ecotoxicity:

Abiotic degradation: half time (pure a.i.) at 22°C

t _{1/2}	12 d at pH 4
t _{1/2}	29 h at pH 7
t _{1/2}	≤10 min at pH 9

Acute fish toxicity:

LC ₀ for Brachydanio rerio	0.005 mg/l (96 h)
LC ₅₀ for Cyprinus carpio	0.25–0.5 mg/l (96 h)
LC ₅₀ for Carassius auratus	1 mg/l (96 h)
LC ₅₀ for Onchorhynchus mykiss	0.05 mg/l (96 h)
EC ₅₀ for Daphnia magna	~0.57 mg/l (48 h)
IC ₅₀ for algae (Scenedesmus subs.)	> 1 mg/l (72 h)
EC ₅₀ for activated sludge	230 mg/l (OECD test 209)

Oxygene consumption test with Pseudomonas putida: no harmful effects at 125 mg/l.

Antimicrobial effectiveness/applications

In antimicrobial effectiveness Tolyfluanide is very similar to Dichlofluanide (16.5.) It is also, as is Dichlofluanide, light-stable, non-volatile, colourless, virtually insoluble in water and of low toxicity. But the better solubility of Tolyfluanide in organic solvents eases its incorporation into solvent-based wood preservatives, anti-blue-stain agents, primers and wood stains, non-aqueous surface coatings and marine antifouling coatings and makes the microbicide increasingly popular as an alternative to Dichlofluanide.

The MIC's gathered in Table 119 picture Tolyfluanide's spectrum of effectiveness. Its suitability for combating wood rotting fungi is reported by Kühle et al. (1981). – Tolyfluanide is not only effective against fresh water and sea water algae but also against sessile sea organisms. Combinations of Tolyfluanide and copper oxide show excellent performance in antifouling coatings.

Suggested addition rates:

0.4–0.7% in wood stains

1.5–2.5% solvent-based coatings, calculated on film weight

2.5–10% in marine antifouling coatings, calculated on total formulation

Tolyfluanide has been developed by Bayer as a plant protection fungicide; accordingly it is not phytotoxic. Formulations containing the microbicide therefore can be applied on objects which may come into contact with plants after drying.

Microbicide group (substance class)

Chemical name

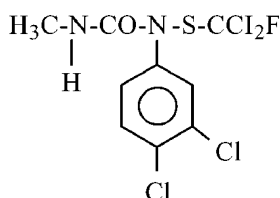
Chemical formula

Structural formula

16. N-HALOALKYLTHIO COMPOUNDS

16.7. N-methyl-N'-3,4-dichlorophenyl-N'-dichlorofluoromethylthiourea

$C_9H_7Cl_4FN_2OS$



352.05

88308-77-4

no EC-No.

Molecular mass

CAS-No.

EC-No.

Synonym/common name

N-(dichlorofluoromethylthio)-N-(methylcarbamoyl)-(3,4-dichloro)aniline

Table 119 Minimum inhibition concentrations (MIC) of Tolyfluanide in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	10
<i>Aspergillus flavus</i>	75
<i>Aspergillus niger</i>	100
<i>Aspergillus terreus</i>	20
<i>Aspergillus ustus</i>	50
<i>Aureobasidium pullulans</i>	10
<i>Cephaloscyus fragrans hanava</i>	10
<i>Ceratocystis pilifera</i>	10
<i>Chaetomium globosum</i>	20
<i>Cladosporium cladosporioides</i>	35
<i>Cladosporium herbarum</i>	10
<i>Coniophora puteana</i>	10
<i>Fusarium culmorum</i>	20
<i>Fusarium moniliforme</i>	20
<i>Glocladium virens</i>	> 1000
<i>Lentinus tigrinus</i>	5
<i>Mucor racemosus</i>	10
<i>Penicillium glaucum</i>	20
<i>Polyporus versicolor</i>	10
<i>Poria vaporaria</i>	2
<i>Sclerophoma pityophila</i>	10
<i>Trichoderma viride</i>	> 1000
<i>Candida albicans</i>	20
<i>Candida krusei</i>	75
<i>Rhodotorula mucilaginosa</i>	150
<i>Saccharomyces cerevisiae</i>	5
<i>Torula rubra</i>	10
<i>Torula utilis</i>	10
Slime bacteria ^a	2.5
Algae	5–10

^aDescribed by Kato & Fukumura (1962).

Table 120 Minimum inhibition concentrations (MIC) of N-methyl-N'-3,4-dichlorophenyl-N'-dichlorofluoromethylthiourea in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	5
<i>Aspergillus niger</i>	10
<i>Aureobasidium pullulans</i>	10
<i>Chaetomium globosum</i>	5
<i>Cladosporium cladosporioides</i>	5
<i>Coniophora puteana</i>	0.5
<i>Lentinus tigrinus</i>	1
<i>Penicillium glaucum</i>	50
<i>Polyporus versicolor</i>	10
<i>Sclerophoma pityophila</i>	5
<i>Trichoderma viride</i>	100
<i>Candida albicans</i>	50
<i>Candida krusei</i>	35
<i>Rhodotorula mucilaginosa</i>	10
<i>Sporobolomyces roseus</i>	5
<i>Saccharomyces cerevisiae</i>	50
<i>Torula rubra</i>	5
<i>Torula utilis</i>	50
Algae	0.1–1

Chemical and physical properties

Appearance	white crystalline solid
Content (%)	~100
Melting point °C	125–128
Stability	hydrolyzes in aqueous solution (half-life at pH 7.8, 20°C: 208 d); stable in aqueous dispersions, e.g. in water based paint formulations at pH 8.5–9 for longer than 1 year at room temperature
Solubility g/l	500 in acetone, 300 in ethyl acetate, 60 in xylene, 1.0 in white spirit; virtually insoluble in H ₂ O

Toxicity data (source: BAYER)

LD ₅₀ oral	approx. 650–1650 mg/kg rat
Irritant to skin and mucosa.	

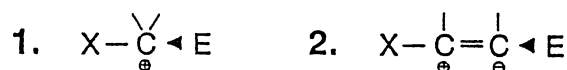
The toxicological properties of the microbicide were not yet completely examined.

Antimicrobial effectiveness/applications

This sulphenylated urea derivative, which was not detected before 1985 (Kühle et al.) occupies a very special position within the range of N-haloalkylthio compounds because of its stability coupled with high antimicrobial activity and a broad spectrum of effectiveness (see Table 120). The starting material in the synthesis of the sulphenylated urea derivative is N-methyl-N'-3,4-dichlorophenylurea, a potent photosynthesis inhibitor (similar to Diuron, 10.9.) and consequently active as an algicide. But the transfer of the S-CCl₂F group to the urea derivative leads to a considerable increase in algicidal activity and additionally equips the molecule with strong fungicidal efficacy. With regard to the use of N-methyl-N'-3,4-dichlorophenyl-N'-dichlorofluoromethylthiourea as an algicide in antifouling coatings it is remarkable that even after a hydrolytic cleavage of the S-CCl₂F group the antialgal activity is not lost, as the remaining N-methyl-N'-3,4-dichlorophenylurea exhibits algicidal efficacy too. The chemical and physical properties of the sulphenylated urea derivative predispose the microbicide for the fungicidal and algicidal treatment of surface coatings, however, the further development of the product is uncertain.

17. Compounds with activated halogen atoms

Microbicides of this kind are electrophilic active substances having at their disposal an activated halogen atom in the α -position and/or in the vinyl position to an electronegative group E (Figure 20). The antimicrobial activity of these substances arises from the fact that nucleophilic entities (H-Nu) of the microbial cell react with the carbon atom boasting an electron hole. It is apparent that a variety of modifications of both the electronegative



X = halogen

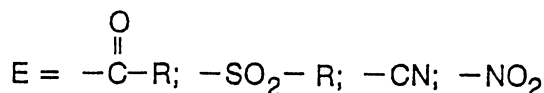


Figure 20 Activated halogen atoms.

group E and the portion of the molecule bearing the halogen atom are possible. Thus microbicides having a wide range of chemical and physical properties have been synthesized within this group of active ingredients. See also Part I, Chapter 2 (Figure 11).

The following microbicides with activated halogen atoms have already been described within other substance classes:

under 3.4. AMIDE-FORMALDEHYDE-REACTION-PRODUCTS

3.4.1. N-Hydroxymethyl-chloracetamide

3.4.2. 2,2,3-Trichloro-N-hydroxymethyl-propionamide

under 9. ACID ESTERS

9.2. 2-Bromo-ethylacetate

9.3. 2-Bromo-benzylacetate

9.4. Bis-1,2-(bromoacetoxy)ethane

9.5. Bis-1,4-(bromoacetoxy)-2-butene

9.6. (2-Bromo-1,2-diiodoacryl-)ethylcarbonate

under 15. HETEROCYCLIC N,S COMPOUNDS

15.2. 5-Chloro-2-methyl-4-isothiazolin-3-one (CMI)

15.5. 4,5-Dichloro-2-(n-octyl)-4-isothiazolin-3-one (DCOI)

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.1. 2-Chloroacetamide
Chemical formula	C ₂ H ₄ ClNO
Structural formula	Cl-CH ₂ -CO-NH ₂
Molecular mass	93.51
CAS-No.	79-07-2
EC-No.	201-174-2; EEC-no. 41
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	alpha-chloroacetamide
Supplier	RIEDEL DE HAEN, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless crystals, odourless
Content (%)	~100
Boiling point/range °C (98 kPa)	224 (decomposition)
Melting point °C	119 – 120
Vapour pressure hPa (20°C)	0.07
Flash point °C	170
pH (1% in H ₂ O)	4.5
Stability	saponification/inactivation in strong acids and alkalis; relatively stable between pH 4 and 9; half-life at pH 8: 2600 h = 108 days

Solubility g/l (20°C)	approx. 50 in H ₂ O (at 40°C approx. 150), approx. 100 in ethanol; soluble in ether
Toxicity data	
LD ₅₀ oral	138 mg/kg rat 155 mg/kg mouse 31 mg/kg dog
LD ₅₀ intraperitoneal	100 mg/kg mouse
LD ₅₀ intravenous	180 mg/kg mouse
Moderately irritant to skin and mucosa, contact dermatitis possible; potential skin sensitizer. Not mutagenic (Ames test, micronucleus test, dominant lethal test negative).	
Ecotoxicity (source: BAYER):	
LC ₅₀ for <i>Lebistes reticulans</i>	10 mg/l (10 days)
Tolerated concentration by activated sludge organisms:	150 mg/l

Antimicrobial effectiveness/applications

Among the three haloacetamides, chloro-, bromo- and iodoacetamide, 2-chloroacetamide is the one with the lowest antimicrobial efficacy, although it is the stronger electrophilic compound because of the high electronegativity of the chloro atom (see Table 83).

But its lipid solubility is much lower than that of bromo- and iodoacetamide. Comparison data of the three 2-halo-acetamides are listed in Table 121. Nevertheless 2-chloroacetamide only has gained significant importance as a preservative for the protection of aqueous functional fluids because of its good water solubility and in consequence its favourable partition coefficient. Other advantages of 2-chloroacetamide are the following properties: colourless, odourless, effective over a wide range of pH values (4–9), compatible with anionic and non-ionic compounds. The antimicrobial activity increases even in the presence of anionic detergents. The activity of the 2-chloroacetamide against mould producing fungi is more distinctive than the activity against bacteria, however in total, 2-chloroacetamide cannot be listed among the highly effective microbicides (see Table 122).

Accordingly the addition rates are relatively high; they vary between 0.2 and 0.6% for the in-can protection of water based paints, adhesives, glues and casein. 2-Chloroacetamide may also be used as a preserving agent in the leather industry; for the protection of wet blues one needs approx. 0.2–2% active ingredient calculated on pelt weight. In the EEC list of preservatives which are permitted for use in cosmetic products, 2-chloroacetamide is mentioned (No. 41) with a maximum authorized concentration of 0.3% and a printed warning label: “contains chloroacetamide”. Percentage of use in US formulations: 0.41%.

Table 121 Minimum inhibition concentration (MIC), M_r , and half-life ($t_{1/2}$) of 2-halo-acetamides

Halo-acetamide	MIC (mg/litre) and mM/litre) against		M_r	$t_{1/2}$ at pH 8 (h)
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>		
2-Chloro-acetamide	2000/21	1500/16	93.51	2600
2-Bromo-acetamide	200/1.5	100/0.75	137.97	5000
2-Iodo-acetamide	50/0.27	20/0.11	184.96	

Table 122 Minimum inhibition concentrations (MIC) of 2-chloroacetamide in nutrient agar

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	5000
<i>Aeromonas punctata</i>	2000
<i>Bacillus subtilis</i>	5000
<i>Escherichia coli</i>	2000
<i>Proteus vulgaris</i>	5000
<i>Pseudomonas aeruginosa</i>	1500
<i>Pseudomonas fluorescens</i>	5000
<i>Staphylococcus aureus</i>	1500
<i>Alternaria alternata</i>	100
<i>Aspergillus niger</i>	800
<i>Aureobasidium pullulans</i>	750

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.2. 2-Bromoacetamide
Chemical formula	C ₂ H ₄ BrNO
Structural formula	Br-CH ₂ -CO-NH ₂
Molecular mass	137.97
CAS-No.	683-57-8
EC-No.	unknown
Synonym/common name	alpha-bromoacetamide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	~100
Melting point °C	102 – 105
Stability	sensitive to light and heat; hydrolytic cleavage in acids and alkalis; relatively stable in water between pH 4 and 9; half-life at pH 8: 5000 h = 208 days
Solubility	sparingly soluble in H ₂ O, soluble in organic solvents
Toxicity data	
LD ₅₀ oral	100 mg/kg rat 124 mg/kg mouse 61 mg/kg rabbit
LD ₅₀ dermal	3160 mg/kg rat
In tests with rabbits 500 mg 2-bromoacetamide (4 h) caused severe skin irritation; 100 mg caused severe eye irritation.	
LC ₅₀ for fish	12.1 mg/l

Antimicrobial effectiveness/applications

2-Bromoacetamide exhibits a broad and equalized spectrum of activity, somewhat more distinctive against fungi than against bacteria (see Tables 121 and 123). 5–10 mg bromoacetamide/litre are sufficient to inhibit the growth of slime forming bacteria, e.g. those which can grow by utilizing ϵ -caprolactam or its cyclic dimer or trimer as the sole carbon source (Kato & Fukumura, 1962). 2-Bromoacetamide exhibits antimicrobial activity also via the vapour phase. However, up to now bromoacetamide has no significant importance as a preserving agent probably because of toxicity and cost reasons. – Examinations performed by the Institute for Parasitologie (Chin. Acad. med., 1981) have shown that 2-bromoacetamide disposes of strong molluscicidal efficacy.

Table 123 Minimum inhibition concentrations (MIC) of 2-bromoacetamide in nutrient agar against fungi

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	20
<i>Aspergillus niger</i>	50
<i>Aureobasidium pullulans</i>	50
<i>Chaetomium globosum</i>	20
<i>Coniophora puteana</i>	5
<i>Lentinus tigrinus</i>	10
<i>Penicillium glaucum</i>	50
<i>Polyporus versicolor</i>	50
<i>Sclerophoma pityophila</i>	20
<i>Trichoderma viride</i>	100

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.3. 2-Iodoacetamide
Chemical formula	C ₂ H ₄ INO
Structural formula	I-CH ₂ -CO-NH ₂

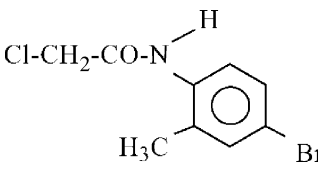
Molecular mass	184.96
CAS-No.	144-48-9
EC-No.	205-630-1
EPA TSCA	Section 8 (B) Chemical Inventory
Synonym/common name	alpha-iodoacetamide
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless crystals
Content (%)	min. 99
Melting point °C	91–93
Stability	light sensitive; hydrolytic cleavage in strong acids and alkalis
Solubility	sparingly soluble in H ₂ O, highly soluble in organic solvents
Toxicity data	
LD50 oral	74 mg/kg mouse
intraperitoneal	50 mg/kg mouse
intravenous	56 mg/kg mouse
Equivocal tumorigenic agent by RTECS criteria.	

Antimicrobial effectiveness/application

The toxic 2-iodoacetamide has no practical importance as a microbicide for the protection of materials and is mentioned here for comparison purposes, only.

Table 124 Minimum inhibition concentrations (MIC) of 2-iodoacetamide in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	500
<i>Chaetomium globosum</i>	200
<i>Penicillium glaucum</i>	100
<i>Rhizopus nigricans</i>	200
<i>Escherichia coli</i>	50
<i>Pseudomonas aeruginosa</i>	20

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.4. N-(4-Bromo-2-methylphenyl)-2-chloroacetamide (BMPCA)
Chemical formula	C ₉ H ₉ BrClNO
Structural formula	
Molecular mass	262.54
CAS-No.	96686-51-0
Synonym/common name	2-chloroacet(2-methyl-4-bromo)anilide
Supplier	COSAN CHEM. CORP.
Chemical and physical properties	
Appearance	white, odourless powder
Content (%)	~100

Melting point °C	133–135
Vapour pressure hPa (24°C)	<2
Stability	sensitive to strong acids and alkalis (saponification); stable in aqueous surface coatings
Solubility	practically insoluble in H ₂ O, soluble in organic solvents
Toxicity data (source: COSAN)	
LD ₅₀ oral	4044 mg/kg rat
dermal	>21 g/kg rabbit
LC ₅₀ on inhalation	>10 mg/l for rats

No sensitization in the guinea pig test. Slightly to moderately irritant to skin and mucosa. Mutagenicity/carcinogenicity tests negative (Dalton, 1988).

Antimicrobial effectiveness/application

The antimicrobial activity of BMPCA is especially directed against a broad spectrum of fungi (see Table 125). As it is stable in aqueous formulations at pH values up to nine, not significantly leachable, non-volatile, not causing colorations (resistant to photo-oxidation), it has been recommended for use as a paint film fungicide (Dalton, 1988).

Table 125 Minimum inhibition concentrations (MIC) of BMPCA in nutrient solution

Test organism	MIC (mg/litre)
<i>Alternaria solani</i>	31
<i>Aspergillus niger</i>	31
<i>Aureobasidium pullulans</i>	63
<i>Chaetomium globosum</i>	31
<i>Cladosporium cladosporioides</i>	50
<i>Neurospora species</i>	125
<i>Penicillium glaucum</i>	50
<i>Sclerophoma pityophila</i>	50

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.5. 2,2-Dibromo-3-nitrilopropionamide (DBNPA)
Chemical formula	C ₃ H ₂ Br ₂ N ₂ O
Structural formula	NC-CBr ₂ -CO-NH ₂
Molecular mass	241.86
CAS-No.	10222-01-2
EC-No.	233-539-7
EPA Reg.	approval for antimicrobial applications
Synonym/common name	2,2-dibromo-2-cyanoacetamide
Supplier	BUCKMAN, DOW, THOR
Chemical and physical properties	
Appearance	free-flowing white crystalline solid
Content (%)	~99
Melting point °C	124–126
Density g/ml (20°C)	1.4–1.6
Vapour pressure hPa (20°C)	2.9 × 10 ⁻⁵
Log POW	1.01

Stability	thermal decomposition starts at 186°C; rapid decomposition/hydrolysis in aqueous media at pH ≥ 7 ; inactivation by strong reducing agents and/or nucleophilic reagents (sulphur containing nucleophiles, including H ₂ S, sulphites, bisulphites and compounds containing thiol groups, e.g. 2-mercaptobenzthiazole (15.10.); not compatible with the microbicide Dazomet (3.3.25.); sensitive to sunlight (photodegradation); relatively stable in aqueous acidic media in the presence of stabilizers such as urea derivatives, caprolactam, dimethylhydantoin and others (Burk & Reineke, 1979); unstable in dimethyl sulphoxide and dimethyl formamide
Solubility g/l	15 in H ₂ O, 250 in ethanol, 90 in ethylene glycol, 200 in propylene glycol, 350 in isopropanol, 500 in methyl ethyl ketone
Toxicity data (source: DOW)	
LD ₅₀ oral	167–235 mg/kg rat
LD ₅₀ dermal	>2000 mg/kg rabbit
LC ₅₀ on inhalation (fine dust, nose-only exposure):	0.24 mg/l for female rats (4 h) 0.31 mg/l for male rats (4 h)
DBNPA may cause severe skin and eye irritation (with corneal injury); the inhalation of dust irritates the upper respiratory tract.	
In animal studies DBNPA has been shown not to interfere reproduction. Mutagenicity studies (in vitro and with animals) were negative.	
Ecotoxicity: DBNPA will degrade rapidly in the environment by chemical reactions: hydrolysis, oxidation, reduction.	
LC ₅₀ for activated sludge	3.1 mg/l (OECD Test No. 209)
LC ₅₀ for fish: Fathead minnow	1.8–2.2 mg/l
Rainbow trout	0.71–2.0 mg/l
LC ₅₀ for Daphnia magna	0.732 mg/l
IC ₅₀ for green algae: Selenastrum capricornutum	0.30 mg/l (72 h)

Antimicrobial effectiveness/applications

DBNPA's spectrum of efficacy is broad and equalized (see Table 126). It covers, Gram-positive and Gram-negative bacteria, yeast, fungi and algae. In particular remarkable is its effectiveness against slime forming micro-organisms, e.g. those described by Kato & Fukumura (1962) which are inhibited by 0.5–1 mg/l. Due to its very distinct electrophilic character DBNPA exhibits fast antimicrobial action by reactions with nucleophilic cell compounds such as the protein fractions of the cell membrane and enzyme systems. The rapidity of DBNPA's action requires that one adds the active component to systems already containing micro-organisms.

Rates of hydrolysis of DBNPA at different pH values were determined by Exner et al. (1973) and summarized in Table 127.

The decomposition pathways of DBNPA described by Exner et al. (1973) are shown in Figure 21. Hydrolysis ends with oxalic acid which oxidizes slowly to CO₂. The reaction of DBNPA with nucleophiles leads to malonic acid, which is just as oxalic acid a naturally occurring dicarboxylic acid; it may further degrade to acetic acid and CO₂.

One can characterize DBNPA as a potent but not persistent microbicide the application of which does not cause waste water problems. Formulations containing 40%, 20% or 5% a.i., are available. They may be used to inhibit biofouling in recirculating water (cooling towers) and once-through industrial water systems, in pulp, paper and paperboard mills, in enhanced oil recovery systems, in aqueous metalworking fluids. As a preservative DBNPA is efficient only for the short term protection of aqueous products.

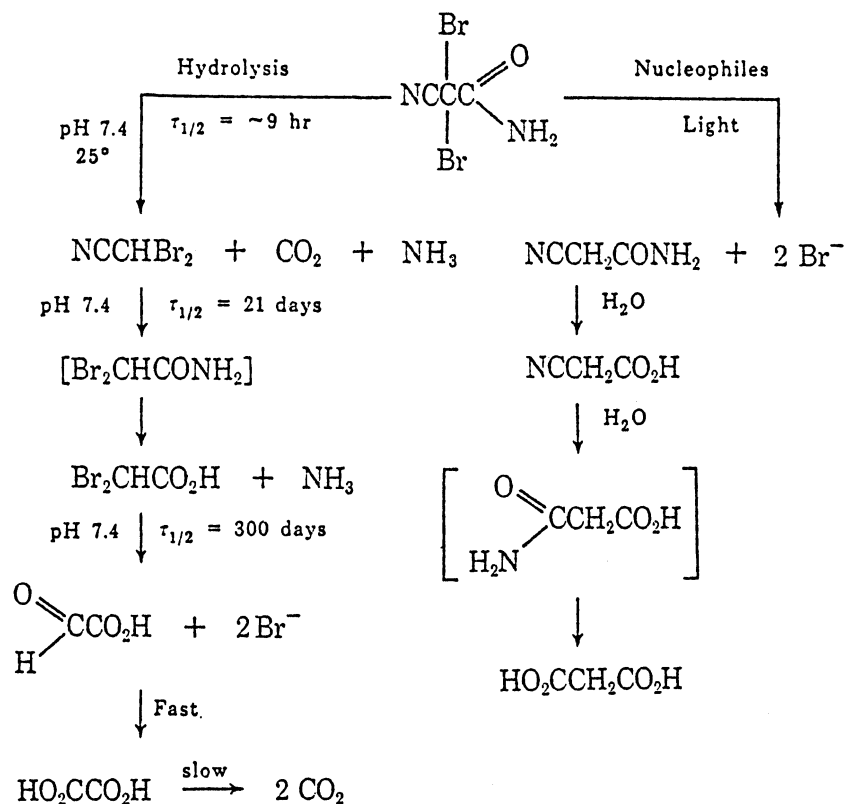
With regard to the treatment of cooling water it is worth mentioning that DBNPA is compatible with oxidizing microbicides (21.); together with chlorine it exhibits remarkable synergistic performance.

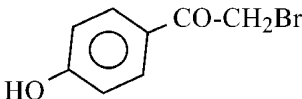
Table 126 Minimum inhibition concentrations (MIC in mg/l) of DBNPA in nutrient agar (Source: DOW)

Organism	ATCC#	pH 5.5 Solution
Fungi		
<i>Aspergillus niger</i>	16404	1250
<i>Fusarium oxysporum</i>	48112	1250
<i>Penicillium chrysogenum</i>	9480	1250
<i>Pullularia pullulans</i>	16622	1250
<i>Trichoderma viride</i>	8678	1250
Yeast		
<i>Candida albicans</i>	10231	1250
<i>Saccharomyces cerevisiae</i>	4105	500
Bacteria		
<i>Bacillus subtilis</i>	8473	125
<i>Enterobacter aerogenes</i>	13048	125
<i>Escherichia coli</i>	11229	125
<i>Klebsiella pneumoniae</i>	8308	125
<i>Proteus vulgaris</i>	881	125
<i>Pseudomonas aeruginosa</i>	10145	125
<i>Pseudomonas aeruginosa</i> PRD10	15442	125
<i>Salmonella choleraesuis</i>	10708	125
<i>Staphylococcus aureus</i>	6538	125

Table 127 Rates of hydrolysis of DBNPA

PH	$t_{1/2}$ (h)	Temp. (°C)
3.9	2140	23
6.0	155	25
6.7	37.0	25
7.3	8.8	25
7.7	5.8	25
7.7	145	0
8.0	2.0	25
8.9	0.34	25
9.7	0.11	25
9.7	1.5	0

**Figure 21** Decomposition pathways of DBNPA

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.6. 2-Bromo-4'-hydroxyacetophenone
Chemical formula	$C_8H_7BrO_2$
Structural formula	
Molecular mass	215.05
CAS-No.	2491-38-5
EC-No.	219-665-0
Synonym/common name	4-(bromoacetyl)phenol
Supplier	BUCKMAN
Chemical and physical properties	
Appearance	violet coloured crystals
Content (%)	~100
Melting point °C	127–129
Stability	hydrolyzes slowly in aqueous media (increasingly with increasing pH and temperature)
Solubility	sparingly in H_2O , soluble in organic solvents
Chemical and physical properties of a 10% formulation	
Appearance	brownish-red liquid with a slight odour
Boiling point °C (101 kPa)	>115
Solidification point °C	–25
Density g/ml (25°C)	1.02
Viscosity mPa·s	<10
Flash point °C	70 (closed cup)
Solubility	miscible with water
Toxicity data of the 10% formulation (source: BUCKMAN)	
LD ₅₀ oral	1150 mg/kg rat
LD ₅₀ dermal	>2000 mg/kg rat
Corrosive to skin, eyes and mucosa.	
Skin sensitizer.	
Ecotoxicity: The concentrated product is toxic to fish.	

Antimicrobial effectiveness/application

The microbicide has been used as an active ingredient in slimicides for the application in process water systems, predominantly in pulp and paper mills. In the meantime it has been widely substituted by microbicides which are safer with regard to their toxicity.

Table 128 Minimum inhibition concentration (MIC) of 4-(bromoacetyl) phenol

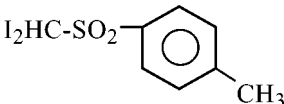
Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	100
<i>Chaetomium globosum</i>	500
<i>Penicillium glaucum</i>	200
<i>Escherichia coli</i>	100
<i>Pseudomonas aeruginosa</i>	200
Slime bacteria ^a	1–2

^aDescribed by Kato & Fukumura (1962).

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.7. Bis(trichloromethyl)sulphone
Chemical formula	$C_2Cl_6O_2S$
Structural formula	$Cl_3C-SO_2-CCl_3$
Molecular mass	300.81
CAS-No.	3064-70-8
EC-No.	221-310-4
FDA Approval	as slimicide for the manufacture of paper and paper board in contact with food (CFR No. 176.300)
Synonym/common name	hexachlorodimethylsulphone
Supplier	LAMIRSA, STAUFFER CHEM.
Chemical and physical properties	
Appearance	white crystalline powder with a pungent odour (lachrymatory)
Content (%)	min. 97
Melting point °C	35–38
Stability	thermal decomposition starts at 100–140°C; hydrolyzes in aqueous media of pH >7
Solubility g/l	0.07 in H_2O , 780 in benzene, 530 in hexane, 450 in Solvesso 150, 600 in white spirit, 150 in linseed oil
Toxicity data (source: LAMIRSA)	
LD ₅₀ oral	708 mg/kg rat
LD ₅₀ dermal	5620 mg/kg rabbit
Irritant/corrosive to skin and mucosa.	

Antimicrobial effectiveness/applications

Hexachlorodimethylsulphone is a reactive microbicide which exhibits a broad spectrum of activity. The minimum inhibition concentrations for bacteria, fungi, yeasts and algae range between 10 and 100 mg/litre. The slime forming bacteria described by Kato & Fukumura (1962) are especially sensitive to hexachlorodimethylsulphone; MIC: 0.5 mg/litre. In consequence of its limited stability in water the compound is mainly used as a non-persistent slimicide and algicide in process water circuits, e.g. in the paper industry, and not so much as a preservative.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.8. p-[(Diiodomethyl)sulphonyl]toluene
Chemical formula	$C_8H_8I_2O_2S$
Structural formula	
Molecular mass	422.03
CAS-No.	20018-09-1
EC-No.	243-468-3
EPA Reg.	approval for different antimicrobial applications
FDA	clearances for several direct food contact applications
Synonym/common name	diiodomethyl-p-tolylsulphone
Supplier	DOW/ANGUS
Chemical and physical properties	
Appearance	tan fine powder with a slight acidic odour
Content (%)	>90
Melting point °C	157

Density g/ml (25°C)	1.4
Log POW	462
pH of a suspension (10 g/l) in H ₂ O	5–7
Stability	stable and effective through the pH range 4–10; sensitive to strong alkalis, thermal decomposition at approx. 200°C
Solubility g/l (25°C)	0.0001 in H ₂ O, 20 ethanol, 10 in isopropyl alcohol, 350 in acetone, 25 in methyl ethyl ketone, 10 in ethylene glycol, 43 in toluene, 33 in xylene, 2 in hexane, 25 in tetrahydrofuran, < 4 in mineral oil

Toxicity data (source: ANGUS)

LD ₅₀ oral	>9400 mg/kg rat > 10000 mg/kg mouse
LD ₅₀ dermal	>2000 mg/kg rabbit
LC ₅₀ on inhalation	0.96 mg/l (4 h) for rats

In tests with rabbits not irritant to the skin, however, risk of serious damage to the eyes.

The microbicide is judged to be non-sensitizing according to the guinea pig test (Draize test). – In a series of in-vitro and in-vivo assays it did not demonstrate mutagenic activity. – Teratogenicity studies (oral application, 6 to 15 days); no-reproductive-effect level: 4 mg/kg/day for rabbits and 1000 mg/kg/day for rats.

Ecotoxicity:

Diiodomethyl-p-tolylsulphone does not persist in the environment. It has been found that degradation followed the pathway whereby the iodine moieties are lost to ultimately yield p-toluenesulphonic acid.

Acute toxicity to aquatic animals:

LC ₅₀ for Bluegill sunfish	0.13 mg/l (96 h)
for Rainbow trout	0.75 mg/l (96 h)
LC ₅₀ for <i>Daphnia magna</i>	8 mg/l (96 h)

Toxicity to *Pseudomonas putida*: EC₁₀ > 10000 mg/l

Antimicrobial effectiveness/applications

Diiodomethyl-p-tolylsulphone can be regarded as a broad spectrum microbicide which is more active against fungi, yeasts and algae than against bacteria. Being not volatile and practically insoluble in water it is recommended for the fungicidal treatment of aqueous or solvent based coatings, adhesives, sealants, etc., for interior or exterior applications. As an aliphatic iodo compound it can cause some yellow coloration, especially in white coatings. The addition of colour suppressants to the active ingredient can alleviate the coloration problem. It is also recommended to apply diiodomethyl-p-tolylsulphone in the tanning process for the protection of wet blues

Table 129 Minimum inhibition concentrations (MIC) of diiodomethyl-p-toyl-sulphone in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	3.5
<i>Aspergillus niger</i>	5
<i>Aureobasidium pullulans</i>	7.5
<i>Chaetomium globosum</i>	5
<i>Cladosporium cladosporioides</i>	1
<i>Lentinus tigrinus</i>	5
<i>Penicillium glaucum</i>	5
<i>Rhizopus nigricans</i>	100
<i>Sclerophoma pityophila</i>	1
<i>Trichoderma viride</i>	10
<i>Candida albicans</i>	20
<i>Candida krusei</i>	50
<i>Rhodotorula mucilaginosa</i>	10
<i>Saccharomyces bailii</i>	5
<i>Saccharomyces cerevisiae</i>	10
<i>Torula rubra</i>	10
<i>Torula utilis</i>	10
<i>Escherichia coli</i>	200
<i>Proteus vulgaris</i>	> 1000
<i>Pseudomonas aeruginosa</i>	> 1000
<i>Staphylococcus aureus</i>	50

against mould infestation. It has been found effective as a preservative for wood, particularly for the prevention of sap stain. Other proposals are: application of diiodomethyl-p-tolylsulphone in metal working fluids and in pulp and paper mills to overcome problems caused by fungal growth.

Formulations of the microbicide adapted to various applications are available: a water based dispersion containing ~40% a. i. and a wettable powder containing ~46% a. i.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility g/l (25°C)

Toxicity data (source: ANGUS)

LD₅₀ oral

In tests with rabbits not irritant to the skin, but irritant to the eyes.

Ecotoxicity:

Toxic to aquatic organisms.

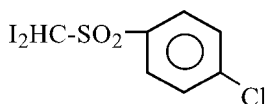
LC₅₀ for Rainbow trout

for Bluegill sunfish

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

17.9. p-[(Diiodomethyl)sulphonyl]-chlorobenzene

C₇H₅ClI₂O₂S



442.45

20018-12-6

no EC-no.

4-chlorophenyl-diiodomethylsulphone

fine tan powder

> 90

134–138

stable between pH 4 and 10

0.0002 in H₂O, 40 in ethanol, 20 in isopropanol, 20 in propylene glycol, 350 in acetone, 95 in toluene

3600 mg/kg mouse

600 mg/kg rat

Antimicrobial effectiveness/applications

4-Chlorophenyl-diiodomethylsulphone may be used as is reported for diiodomethyl-p-tolylsulphone (17.8) which is, however, the preferred microbicide.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

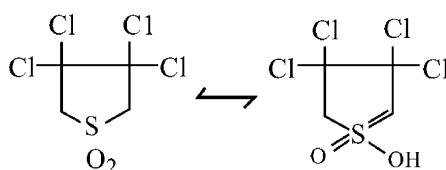
CAS-No.

EC-No.

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

17.10. 3,3,4,4-Tetrachloro-tetrahydro-1,1-dioxo-thiophene

C₄H₄Cl₄O₂S



257.95

3737-41-5

unknown

Synonym/common name	3,3,4,4-tetrachloro-sulpholane
Supplier	LAMIRSA
Chemical and physical properties	
Appearance	white powder with a pungent odour
Content (%)	min. 97
Melting point °C	65–68
pH of a 1% dispersion in H ₂ O	6.6
Stability	acid stable, hydrolysis/decomposition at pH values > 8
Solubility g/l	100 in methanol, 50 in ethanol, 20 in propylene glycol, 330 in acetone, 80 in toluene; sparingly soluble in H ₂ O
Toxicity data (source: LAMIRSA)	
LD ₅₀ oral	250 mg/kg rat
dermal	8700 mg/kg rabbit
Irritant to the skin, corrosive to mucous membranes at 5% in methanol.	

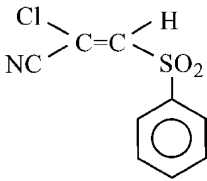
Antimicrobial effectiveness/applications

The reactive compound is effective against all kinds of bacteria, aerobic and anaerobic, and against fungi and yeasts.

Minimum inhibition concentrations

For bacteria	25–100 mg/litre
For fungi	5–10 mg/litre

The microbicide is used as an active ingredient for the formulation of non-persistent slimicides to be applied in paper mills and cooling water systems. In neutral to acid aqueous functional fluids it may also be used as a preservative.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.11. (2-Chloro-2-cyanovinyl)-phenylsulphone
Chemical formula	C ₉ H ₆ ClNO ₂ S
Structural formula	
Molecular mass	227.67
CAS-No.	60736-58-5
EC-No.	262-395-8
Synonym/common name	1-chloro-1-cyano-2-phenylsulphonylethylene
Supplier	BAYER

Chemical and physical properties of a glycolic formulation

Appearance	yellowish to brown liquid with a faint aromatic odour
Content (%)	9.5–10.5 (contains 3% citric acid)
Boiling point/range °C (101 kPa)	~150
Solidification point °C	~-50
Density g/ml (20°C)	~1.16
Vapour pressure hPa (20°C)	< 0.01
Viscosity mPa·s (25°C)	~74
Refractive index nD (20°C)	1.475
Flash point °C	~160
Ignition temperature °C	~390
pH (0.1% in H ₂ O)	~4

Stability	hydrolysis and inactivation in aqueous media increasingly with increasing pH and temperature
Solubility	emulsifiable in water, highly soluble in organic solvents

Toxicity data (source: BAYER)

LD₅₀ oral >5000 mg/kg rat

In tests with rabbits the product caused moderate skin and eye irritation (exposure: 24 h). Inhalation hazard test (dynamic vaporization, 7 h) with rats: no mortality.

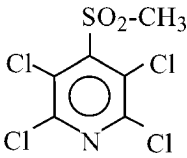
Ecotoxicity:

The a.i. is degradable (96% within 24 h); application concentrations of the formulation have no adverse effects on the degradation activity of biological sewage plants. EC₅₀ for activated sludge approx. 9.2 mg/l. – The a.i. is toxic to fish at a concentration of 1 mg/l. The LC₅₀ of the hydrolysis product (phenylsulphonylacetonitrile) for *Leuciscus idus* is more than 50 mg/l (exposure: 96 h).

Antimicrobial effectiveness/application

(2-Chloro-2-cyanovinyl)-phenylsulphone is active against bacteria, fungi and yeasts. The minimum inhibition concentrations of the formulation range between 5 and 30 mg/l; especially toxic is the formulation to slime forming micro-organisms such as characterized by Kato & Fukumura (1962); MIC: < 0.5 mg/l nutrient solution. Accordingly the microbicide is used as an active ingredient for the formulation of non persistent slimicides to be used in paper mills and cooling water circuits. The formulation described here must not be pre-diluted with water or other protic solvents (such as alcohols) since there is a risk of decomposition, with the a.i. degrading hydrolytically into inactive substances.

Appropriate additions of the formulation range from 10 to 30 mg/l water volume to be protected.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.12. 2,3,5,6-Tetrachloro-4-(methylsulphonyl)pyridine
Chemical formula	C ₆ H ₃ Cl ₄ NO ₂ S
Structural formula	
Molecular mass	294.28
CAS-No.	13108-52-6
EC-No.	236-035-5
Synonym/common name	methyl tetrachloropyridin-4-yl sulphone
Supplier	formerly DOW, ICI
Chemical and physical properties	
Appearance	off-white to fawn crystalline powder
Content (%)	min. 96
Melting point °C	152
Stability	stable and effective over a pH range of 4–8.5; hydrolyzes in alkaline media; negligible degradation on heating to 290°C
Solubility g/l (25°C)	2,5 × 10 ⁻⁵ in H ₂ O, 20 in methanol, 220 in acetone, 343 in DMF, 50 in toluene, < 5 in white spirit
Toxicity data (source: DOW)	
LD ₅₀ oral	1400 mg/kg rat
Irritant to skin and mucosa; can cause skin sensitization.	
Exotoxicity:	
Toxic to aquatic organisms. The microbicide may inhibit biological sewage treatment processes.	

Antimicrobial effectiveness/applications

Tetrachloro-4-(methylsulphonyl)pyridine exhibits a broad spectrum of high antifungal activity and additionally antialgal efficacy. In accordance with its physical and chemical properties it is used as a paint film fungicide and algicide in aqueous and solvent based surface coatings requiring protection from biological growth, in PVC wall coverings, shower curtains, silicone sealants, polypropylene films and wood stains. The addition rates vary between 0.1 and 0.5%. Hydrolysis and inactivation of the active ingredient occurs at a pH in excess of 8.5 and in the presence of organic amines, e.g. morpholine, triethanolamine, diethanolamine; such conditions should be avoided. As far as known the microbicide is no longer available.

Table 130 Minimum inhibition concentrations (MIC) of tetrachloro-4-(methylsulphonyl)pyridine in nutrient agar

Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	7.5
<i>Aspergillus flavus</i>	50
<i>Aspergillus niger</i>	10
<i>Aspergillus terreus</i>	150
<i>Aspergillus ustus</i>	10
<i>Aureobasidium pullulans</i>	7.5
<i>Chaetomium globosum</i>	10
<i>Cladosporium cladosporioides</i>	5
<i>Cladosporium herbarum</i>	10
<i>Coniophora puteana</i>	<1
<i>Paecilomyces variotii</i>	10
<i>Penicillium citrinum</i>	10
<i>Penicillium glaucum</i>	10
<i>Sclerophoma pityophila</i>	5
<i>Stachybotrys chartarum</i>	10
<i>Trichoderma viride</i>	50
<i>Candida albicans</i>	10
<i>Candida krusei</i>	5
<i>Rhodotorula mucilaginosa</i>	10
<i>Saccharomyces bailii</i>	5
<i>Saccharomyces cerevisiae</i>	50
<i>Torula rubra</i>	10
<i>Torula utilis</i>	50
<i>Aerobacter aerogenes</i>	500
<i>Aeromonas punctata</i>	150
<i>Bacillus mycoides</i>	100
<i>Bacillus subtilis</i>	200
<i>Escherichia coli</i>	5000
<i>Pseudomonas aeruginosa</i>	5000
<i>Staphylococcus aureus</i>	50

Microbicide group (substance class)

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

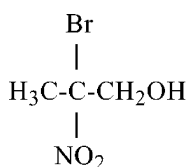
Chemical name

17.13. 2-Bromo-2-nitro-propan-1-ol (BNP)

Chemical formula

$C_3H_6BrNO_3$

Structural formula



Molecular mass

183.99

CAS-No.

24403-04-1

EC-No.

unknown

Synonym/common name

1-bromo-1-hydroxymethyl-1-nitro-ethane

Chemical and physical properties

Appearance

colourless to pale yellow crystals

Content (%)	~100 (HCHO content 16)
Melting point °C	~40
Stability	considerably stable when dry; in water decreasing stability as pH and temperature increase; decomposition products: bromine ions, formaldehyde, nitrite and nitro alcohols; under acid conditions more stable than Bronopol (17.14.)
Solubility g/l	approx. 130 in H ₂ O; highly soluble in polar organic solvents
Toxicity data	
According to nature BNP should show a toxicity profile similar to that of Bronopol (17.14.). That is confirmed by preliminary data.	

Table 131 Minimum inhibition concentration (MIC) of BNP in nutrient agar (Elsmore & Guthrie, 1991)

Test organism	MIC (mg/litre)
Gram-positive bacteria	
<i>Micrococcus flavus</i>	50
<i>Staphylococcus aureus</i>	NCIB 9518 50
<i>Staphylococcus aureus</i>	25
<i>Staphylococcus epidermidis</i>	NCTC 7291 25
<i>Streptococcus faecalis</i>	NCTC 8213 50
Gram-negative bacteria	
<i>Pseudomonas aeruginosa</i>	NCIB 11338 25
<i>Pseudomonas aeruginosa</i> NCTC 6750	50
<i>Pseudomonas putida</i>	NCIB 9034 25
<i>Pseudomonas stutzeri</i>	NCIB 9040 12.5
<i>Pseudomonas cepacia</i>	NCIB 9085 25
<i>Pseudomonas fluorescens</i>	NCIB 9046 25
<i>Pseudomonas</i> sp.	50
<i>Proteus vulgaris</i>	NCTC 4635 25
<i>Proteus morganii</i>	NCTC 10041 25
<i>Escherichia coli</i>	NCTC 5934 25
<i>Escherichia coli</i>	NCTC 9517 25
<i>Klebsiella aerogenes</i>	NCTC 418 25
<i>Enterobacter cloacae</i>	25
<i>Salmonella typhimurium</i>	NCTC 74 25
<i>Serratia marcescens</i>	25
Yeasts	
<i>Candida albicans</i>	ATCC 10231 25–50
<i>Candida tropicalis</i>	25–50
<i>Saccharomyces cerevisiae</i>	NCYC 87 50
<i>Spoilage yeast</i>	50
Spoilage fungi	
<i>Stachybotrys atra</i>	IMI 82021 12.5–25
<i>Myrothecium verrucaria</i>	IMI 45541 50
<i>Amorphotheca resiniae</i>	IMI 89560 200
<i>Aspergillus niger</i>	ATCC 16404 100–200
<i>Aspergillus niger</i>	100–200
<i>Aspergillus</i> sp.	25
<i>Chaetomium globosum</i>	IMI 45550 25–50
<i>Cladosporium herbarum</i>	50
<i>Margarinomyces fasciculatis</i>	50
Spoilage mould	100–200
<i>Penicillium funiculosum</i>	IMI 87160 25–50
<i>Penicillium</i> sp.	50–100
Spoilage mould	200
Spoilage mould	100
Spoilage mould	100–200
<i>Trichoderma viride</i>	400
Algae	
<i>Phormidium foveolarum</i>	CCAP 1462/1 12.5
<i>Nostoc</i> sp.	CCAP 1463/4A 9.4
<i>Chlorella emersonii</i>	CCAP 2211/8A 6.25

Antimicrobial effectiveness/applications

Examinations of Elslmore & Guthrie (1991) have shown that BNP possesses a broad spectrum of activity covering bacteria, yeasts, fungi and algae (see Table 131). The efficacy for fungi is not as distinctive as the activity for bacteria, but by far not as limited as the antifungal activity of Bronopol (17.14). Altogether BNP is more effective than Bronopol which is not surprising considering the fact that BNP bearing only one hydroxymethyl group displays better lipid solubility in comparison to Bronopol bearing two hydroxymethyl groups. The remaining characteristics of BNP are similar to Bronopol; that applies also to the application and fields of application. However, although BNP's significantly enhanced antifungal activity would give additional benefits in various areas of application, it has not displaced Bronopol.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.14 2-Bromo-2-nitropropane-1,3-diol
Chemical formula	$C_3H_6BrNO_4$
Structural formula	$ \begin{array}{c} NO_2 \\ \\ HO-CH_2-C-CH_2-OH \\ \\ Br \end{array} $
Molecular mass	199.99
CAS-No.	52-51-7
EC-No.	200-143-0 – EEC-no.:21
EPA-Reg.	approval for antimicrobial application, TSCA Section 8 (B) Chemical Inventory
FDA	different approvals for applications with food contact and a wide range of other regulatory approvals
Synonym/common name	beta-bromo-beta-nitrotrimethyleneglycol, Bronopol
Supplier	BASF; BAYER; CLARIANT; DOW, THOR
Chemical and physical properties	
Appearance	colourless to pale yellow crystals
Content (%)	~99 (HCHO content 30)
Melting point °C	~130
Density g/ml (20°C)	~1.1
Vapour pressure hPa (20°C)	1.68×10^{-5}
Log POW	0.18
pH (10g/1 H ₂ O)	4.5–7.0
Stability	very stable when dry, slightly hygroscopic; hydrolytic decomposition increases with rising pH and temperature; decomposition products: bromide, nitrite, nitroalkylols and formaldehyde (see Figure 22); in the presence of defined amines and amides nitrite may react to carcinogenic nitrosamines and nitrosamides; deactivation, discolouration and corrosion occur to a limited extent when solutions of Bronopol are in contact with iron and aluminium
Solubility g/l (22–25°C)	250 in H ₂ O, 500 in ethanol, 250 in isopropanol, 140 in propylene glycol, 100 in diethyl sebacate, <5 in oils
Toxicity data (selection)	
LD ₅₀ oral	180 mg/kg rat 270 mg/kg mouse

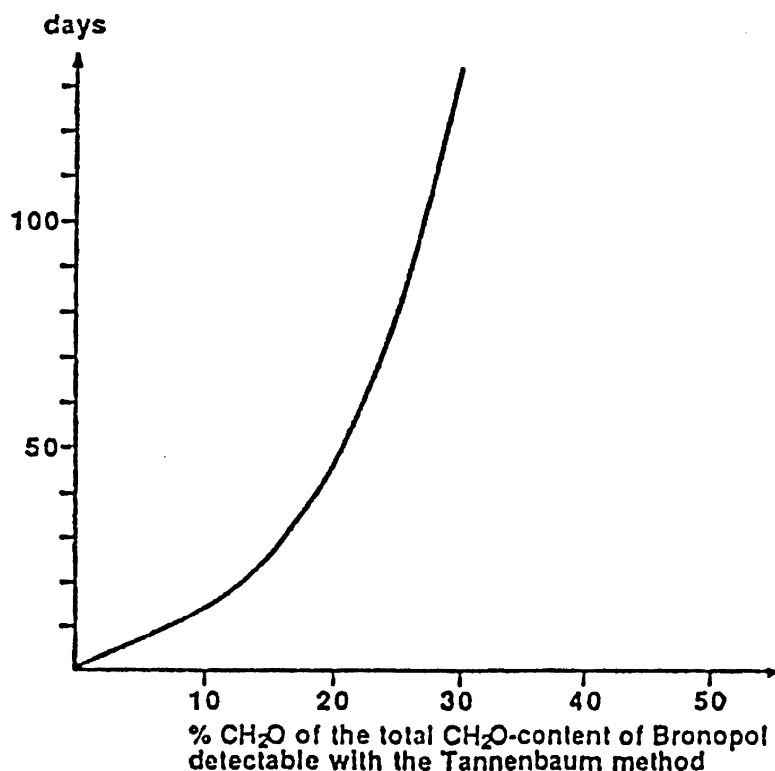


Figure 22 Release of formaldehyde in a 0.1% solution of Bronopol in water at pH 9 (20°C).

LD ₅₀ dermal	1600 mg/kg rat 4750 mg/kg mouse
LD ₅₀ intraperitoneal	26 mg/kg rat 15 mg/kg mouse
LD ₅₀ subcutane	170 mg/kg rat 116 mg/kg mouse
LD ₅₀ intravenous	37.4 mg/kg rat 48 mg/kg mouse
LC ₅₀ on inhalation	>5000 mg/m ³ (6 h) for rats

Irritant to skin, mucosa and eyes. Sensitization is possible by prolonged skin contact. There is no evidence that Bronopol produces carcinogenic, mutagenic, embryotoxic or teratogenic effects.

Ecotoxicity (source: BAYER):

Biological degradation: 50% (test method: Zahn-Wellen; 45 days)

COD value: 600 mg/g

Respiration inhibition of activated sludge organisms 50% at concentrations ≥ 50 mg/l (test according OECD Guideline 209).

LC₅₀ for Rainbow trout 10–100 mg/l (96 h)

EC₅₀ for *Daphnia magna* 1.4 mg/l (48 h)

EC₅₀ for algae (*Scenedesmus subs.*) 0.4–2.8 mg/l (72 h)

Antimicrobial effectiveness/application

Bronopol exhibits a broad spectrum of antimicrobial activity in particular against bacteria, including *Pseudomonads* and sulphate reducing bacteria; the activity against moulds and yeasts is not as distinct (see Table 132). The electrophilic microbicide may react with the microbe cell's nucleophilic centers, e.g. with SH-groups carrying enzymes but also with organic matter. Blood and serum at higher concentrations (50%), thiol compounds such as cysteine, sodium thiosulphate and metabisulphite are markedly antagonistic to the antimicrobial efficacy of Bronopol. The formaldehyde which may release from Bronopol in alkaline media makes a negligible contribution only to the antimicrobial activity of the microbicide.

Bronopol, like the majority of electrophilic active ingredients, has a slow microbicidal effect: Bronopol concentrations 2–4 times higher than the MIC in Table 132 take as long as 24 h to display bactericidal activity.

Table 132 Minimum inhibition concentrations (MIC) of Bronopol (source: BASF)

Test organism	MIC (mg/l)
Gram positive bacteria	
<i>Micrococcus flavus</i> (Product isolate)	25.0
<i>Staphylococcus aureus</i> NCIB 9518	25.0
<i>Staphylococcus epidermidis</i> NCTC 7291	25.0
<i>Streptococcus faecalis</i> NCTC 8213	25.0
Gram negative bacteria	
<i>Escherichia coli</i> NCIB 9517	25.0
<i>Klebsiella aerogenes</i> NCTC 418	25.0
<i>Legionella pneumophila</i> NCTC 11192	50.0
<i>Proteus vulgaris</i> NCTC 4635	25.0
<i>Pseudomonas aeruginosa</i> NCTC 6750	25.0
<i>Burkholderia cepacia</i> NCIB 9085	25.0
<i>Pseudomonas fluorescens</i> NCIB 9046	25.0
<i>Salmonella typhimurium</i> NCTC 74	12.5
<i>Serratia marcescens</i> (Industrial isolate)	12.5
Sulphate reducing bacteria	
<i>Desulphovibrio desulphuricans</i> NCIB 8301	12.5
<i>Desulphovibrio vulgaris</i> NCIB 8303	12.5
Yeasts	
<i>Candida albicans</i> ATCC 10231	1600
<i>Candida pelliculosa</i> (Product isolate)	1600
<i>Candida tropicalis</i> (Industrial isolate)	3200
<i>Saccharomyces cerevisiae</i> NCYC 87	3200
Moulds	
<i>Stachybotrys atra</i> IMI 82021	400
<i>Chaetomium globosum</i> IMI 45550	800
<i>Cladosporium herbarum</i> (Industrial isolate)	1600
<i>Penicillium funiculosum</i> IMI 87160	1600
<i>Aspergillus niger</i> ATCC 16404	3200
<i>Trichoderma viride</i> (Industrial isolate)	6400

Thanks to its characteristics 'colourless and odourless, easily soluble in water and alcohol, low toxicity, good skin compatibility, broad effective spectrum', Bronopol is being used on a large scale as a preservative for cosmetics and pharmaceuticals (concentrations: 0.01–0.1%). It is listed in the EC list of preservatives allowed for the in-can protection of cosmetics (max. authorized concentration: 0.1%; limitations and requirements: avoid formation of nitrosamines); percentage of use in US cosmetic formulations: 1.60%.

Neither anionic nor non-ionic surfactants impair Bronopol's antimicrobial efficacy. For that reason the product is a most suitable preservative for detergent solutions, bath foams, shampoos and hair rinses. Bronopol is also compatible with cationic active ingredients such as benzalkonium chloride (18.1.2.).

Since it is in acidic solutions that Bronopol features the highest stability, weakly acid media are the ideal field of application. This imposes limitations on the applicability of Bronopol as a preservative, especially in alkaline industrial fluids which contain amines able to form N-nitroso compounds, e.g. metal working fluids. To overcome the limited antifungal activity of Bronopol combinations with other actives, e.g. with isothiazolones such as 15.3 and 15.6 are recommended. As a slimicide Bronopol may be used in industrial process water (15–100 mg/l), in pulp and paper mills (10–250 mg/l), in oil field operations (50–100 mg/l).

Further data regarding the activity, properties and safety of Bronopol can be found in the reports of Crowshaw et al. (1964) and Dryce et al. (1978).

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

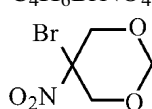
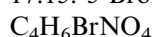
Molecular mass

CAS-NO.

EC-No.

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

17.15. 5-Bromo-5-nitro-1,3-dioxane



212

30007-47-7

250-001-7 – EEC-no.:20

Synonym/common name

Bronidox

Supplier

HENKEL

Chemical and physical properties

Appearance

white crystals

Content (%)

~99 (HCHO content 42.3)

Boiling point/range °C (1.7 kPa)

113–166 (decomposition)

Melting point °C

58–60

Stability

Bronidox is chemically the formal of 2-bromo-2-nitropropane-1,3-diol (Bronopol, 17.14.) and according to its nature more stable than Bronopol; Figure 23 demonstrates that in alkaline media Bronidox liberates formaldehyde (2.1.) in small amounts only and very slowly even at a pH value as high as 9; there is no release of HCHO at pH 7 below 40°C; at pH < 5 and a temperature above 50°C the molecule splits according to retro-aldol-condensation

Solubility g/l (20°C)

4 in H₂O; 250 in ethanol, 100 in isopropanol, 100 in propylene glycol, 500 in trichloro methane; soluble in vegetable oils, insoluble in paraffin oil

Toxicity data (source: Kabara, 1984)

LD₅₀ oral

590 mg/kg mouse

455 mg/kg rat

Skin irritation at concentrations >0.5%; irritation of mucous membranes at concentrations >0.1%. No skin sensitization according to guinea pig test. Bronidox is partly resorbed by the skin.

Antimicrobial effectiveness/application

On account of its affinity with Bronopol, Bronidox too is more effective than one would expect considering its stability and its formaldehyde content. Bronidox has a more equilibrated effective spectrum than Bronopol (see Table 133). It can be used as preservative for functional industrial fluids including cosmetics within a

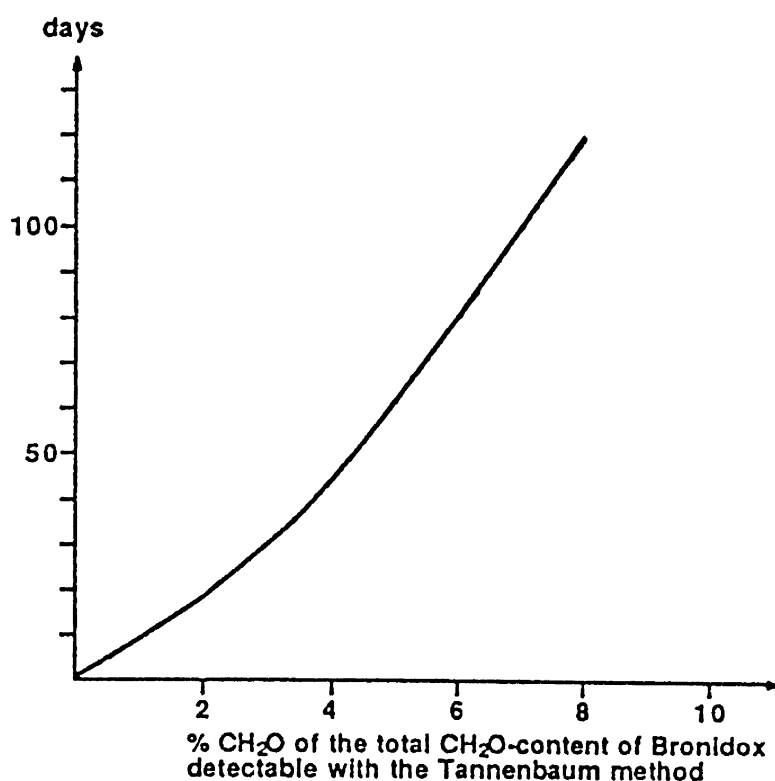


Figure 23 Release of formaldehyde in a 0.1% solution of Bronidox in water at pH 9 (20°C).

Table 133 Minimum inhibition concentrations (MIC) of Bronidox in nutrient agar

Test organism	MIC (mg/litre)
<i>Escherichia coli</i>	35
<i>Proteus vulgaris</i>	50
<i>Pseudomonas aeruginosa</i>	35
<i>Pseudomonas fluorescens</i>	50
<i>Staphylococcus aureus</i>	50
<i>Streptococcus faecalis</i>	75
<i>Candida albicans</i>	50
<i>Aspergillus niger</i>	50
<i>Chaetomium globosum</i>	10
<i>Penicillium glaucum</i>	10

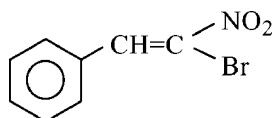
comparatively broad pH range (5–9). The average addition rate is 0.1%. In terms of degradation and potential nitrosamine formation Bronidox and Bronopol behave analogously. Bronidox is mentioned in the EC list of preservatives for cosmetics (max. authorized concentration 0.1%; limitations and requirements: rinse-off products only; avoid formation of nitrosamines). Percentage of use in US cosmetic formulations: 0.29%. The electrophilic microbicide is incompatible with cysteine; slight inactivation occurs in the presence of proteins and reducing agents.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.16. 2,2-Dibromo-2-nitroacetamide
Chemical formula	$C_2H_2Br_2N_2O_3$
Structural formula	$H_2N-CO-CBr_2NO_2$
Molecular mass	261.86
CAS-No.	116920-61-7
EC-No.	unknown
Synonym/common name	$\alpha\alpha$ -dibromonitroacetamide
Supplier	KASEI
Chemical and physical properties	
Appearance	white to yellow monoclinic crystals
Synthesis	treatment of 2-bromo-2-nitroacetamide ammonium salt with bromine in ethanol at 70°C, 15 min or bromination of nitroacetamide sodium salt
Log POW	2.852
pK _a	11.37

Antimicrobial effectiveness/applications

The microbicide is highly effective against bacteria, yeast, fungi and algae and may be used for the control of microorganisms in industrial water systems, paper coatings, polymer emulsions, aqueous paints, adhesives, etc. (Masahiro and Katsuhisa, 2000).

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.17. (2-Bromo-2-nitroethenyl)-benzene
Chemical formula	$C_8H_6BrNO_2$
Structural formula	



Molecular mass	228.05
CAS-No.	7166-19-0
EC-No.	230-515-8 [N106]A
Synonym/common name	(2-bromo-2-nitrovinyl)benzene, 2-bromo-2-nitrostyrene (BNS)
Supplier	BETZ
Chemical and physical properties	
Appearance	yellow crystalline powder
Content (%)	~99
Melting point °C	59–62
Stability	hydrolyzes quickly in aqueous media; reacts under inactivation/detoxification with inorganic or organic sulphite compounds and oxidizing agents such as hydrogen peroxide or potassium permanganate
Solubility	sparingly soluble in water, soluble in organic solvents
Ecotoxicity (source: BETZ)	
Toxic to aquatic animals.	
LC ₅₀ for fish (Rainbow trout, Bluegill sunfish)	1–2 mg/l (96) h

Antimicrobial effectiveness/applications

The cis, trans or mixed forms of BNS are highly effective against a broad spectrum of microbe species. The extraordinarily good activity of BNS against slime bacteria has resulted in the utilization of the compound in compositions useful for inhibiting slime formation in cooling water and pulp and paper mill systems. Due to the natural property of BNS to hydrolyse quickly to less toxic products and the susceptibility of the active ingredient to detoxification by various oxidizing agents it is recommended to use BNS for controlling the fouling potential of molluscs in once-through cooling water systems (Davis & Doherty, 1985).

Table 134 Minimum inhibition concentrations (MIC) of BNS in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	10
<i>Chaetomium globosum</i>	35
<i>Penicillium glaucum</i>	5
<i>Escherichia coli</i>	20
<i>Pseudomonas aeruginosa</i>	10
Slime bacteria ^a	0.25

^aDescribed by Kato & Fukumura (1962).

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.18. 1,2-Dibromo-2,4-dicyanobutane (DCB)
Chemical formula	C ₆ H ₆ Br ₂ N ₂
Structural formula	$ \begin{array}{c} \text{CH}_2\text{-Br} \\ \\ \text{NC-C-CH}_2\text{-CH}_2\text{-CN} \\ \\ \text{Br} \end{array} $
Molecular mass	265.94
CAS-No.	35691-65-7
EC-No.	252-681-0 – EEC-no. 36
FDA approval	21CFR 176.170
Synonym/common name	2-bromo-2-bromomethylglutaronitril
Supplier	CLARIANT-NIPA

Chemical and physical properties

Appearance	white to yellow crystalline powder
Content (%)	98 (2% inert material)
Melting point °C	51–53
Density g/ml (25°C)	2.02
Vapour pressure hPa (20°C)	2.66×10^{-4}
Stability	Stable to approximately 190°C, point of initial decomposition and weight loss (6.5%); sufficiently stable in aqueous formulations up to pH 8 and 60°C
Solubility g/l (R.T.)	3.8 in H ₂ O, 574 in methanol, 147 in ethanol, 73 in ethylene glycol, 1380 in ethylacetate

Toxicity data (source: MERCK, USA)

LD ₅₀ oral	541 mg/kg rat
LD ₅₀ dermal	> 5000 mg/kg rabbit
LC ₅₀ on inhalation	> 200 mg powder/l air (1 h) for rats

In tests with rabbits severe eye irritation.

Irritant to the skin. DCB is not a skin sensitizer.

Ames-Test: negative/non-mutagenic.

Dominant-lethal-Test; no sterility or dominant lethal mutations at feeding levels of 3000 ppm in tests with mice.

Ecotoxicity:

LC ₅₀ for Bluegill sunfish	4.09 mg/l (96 h)
for Rainbow trout	1.75 mg/l (96 h)
for Daphnia magna	2.2 mg/l (48 h)

In activated sludge 99 ppm DCB (highest concentration tested) were completely metabolized within a 24-hour period without change in microorganismen population.

Antimicrobial effectiveness/applications

According to its chemical structure 1,2-dibromo-2,4-dicyanobutane contains several highly electron-withdrawing centres making the compound strongly reactive with nucleophilic groups in the microbial cell. The consequence is a broad activity spectrum covering bacteria, yeasts, fungi and algae (Table 135).

Despite of its poor water solubility DCB has gained considerable importance as a preservative for a wide variety of aqueous functional fluids, such as polymer emulsions, paints, adhesives (including protein based adhesives), mineral slurries, concrete additives, metal working fluids, detergent solutions, cosmetic products, etc., because of its equalized spectrum of effectiveness and its favourable toxicological properties. The EC list of preservatives permitted for use in cosmetic products mentions DCB with a maximum authorized concentration of 0.1%, however, not to be used in cosmetic sunscreen products at a concentration exceeding 0.025%.

Table 135 Minimum inhibition concentrations (MIC) of 1,2-dibromo-2,4-dicyanobutane in nutrient agar (Lederer *et al.*, 1982)

Test organism	MIC (mg/litre)
<i>Aerobacter aerogenes</i>	ATCC 7356 5
<i>Bacillus mycoides</i>	IPC 509 10
<i>Escherichia coli</i>	ATCC 4352 10
<i>Proteus mirabilis</i>	ATCC 7002 10
<i>Pseudomonas aeruginosa</i>	ATCC 10145 100
<i>Pseudomonas</i> sp. (adhesive)	150
<i>Aspergillus niger</i>	ATCC 6275 100
<i>Chaetomium globosum</i>	ATCC 6205 10
<i>Geotrichum</i> sp. (latex emulsion)	100
<i>Penicillium luteum</i>	ATCC 10466 50
<i>Pullularia pullulans</i>	ATCC 9348 25
<i>Trichoderma viride</i>	ATCC 9678 100
<i>Saccharomyces cerevisiae</i>	ATCC 4111 5
<i>Rhodotorula</i> sp. latex emulsion isolate	100
<i>Chlorella pyrenoidosa</i>	Wisc 2005 5
<i>Phormidium imundatum</i>	Wisc 1093 3
<i>Phormidium retzii</i>	Wisc 1094 2

The optimum pH range for a successful application of DCB is 4–8. DCB may interact (inactivation) with iron oxide pigments; in such cases higher concentrations of the preservative are required. In the presence of iron ions there is a risk of yellow discolouration; the problem can be solved by addition chelators, such as EDTA. In alkaline formulations (pH > 8) DCB is highly heat-sensitive. Acidic and neutral products, too should not be heated for a prolonged time. Addition rates: 0.025–0.1%.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA Reg.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (101 kPa)

Melting point °C

Density g/ml (25°C)

Vapour pressure hPa (25°C)

Flash point °C

pH of 10% dispersion in H₂O

Log POW

Stability

Solubility g/l (25°C)

Toxicity data

LD₅₀ oralLD₅₀ dermalLD₅₀ intraperitonealLC₅₀ no inhalation for rats

Non-nutagenic; carcinogenic category 3. Irritant to skin, mucosa and eyes. Non-sensitizing.

Ecotoxicity (source: DEGUSSA):

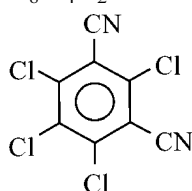
LC₅₀ for Bluegill sunfish
for Rainbow trout

Hazardous for ground waters, water course, sewage system.

Aquatic degradation:

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

17.19. 2,4,5,6-Tetrachloro-1,3-dicyanobenzene

C₈Cl₄N₂

265.91

1897-45-6

217-588-1

for antimicrobial application

Data base, Jan. 2001

2,4,5,6-tetrachloroisophthalonitril, Chlorothalonil

DOW/ANGUS ISP/CREANOVA, THOR

white, crystalline powder with a slight pungent odour
min. 98

> 350 (sublimation)

250–251

1.8

 7.61×10^{-7} (5.97×10^{-5} at 40°C)

not applicable

5–7

2.89

chemically stable to hydrolysis in acidic and neutral water;
hydrolyzes in alkaline solutions increasingly with
increasing pH and temperature; stable to UV radiation;
sublimes when heated above 100°C0.0006 in H₂O, 40 in dimethyl formamide, 65 in linseed oil,
20 in methyl ethyl ketone, 21 in propylene glycol, 70 in
toluene, 80 in xylene

>10000 mg/kg rat

3700 mg/kg mouse

>2500 mg/kg rat

>10000 mg/kg rabbit

2500 mg/kg mouse

310 mg/m³ (1 h)

Antimicrobial effectiveness/applications

Chlorothalonil is especially effective against fungi, yeast and algae. In line with its chemical and physical properties it is used as a non-leachable fungicide in paints, adhesive, sealants, putty, etc. Because of its extremely low solubility in water it is relatively stable also in aqueous paint formulations as far as the pH does not exceed 9. Normal addition levels are in the range 0.25–1%.

Chlorothalonil has demonstrated efficacy not only against algae, but against barnacles, tube worms and other sea animals, too; accordingly it may be applied as an antifouling biocide in marine coatings applied to submerged surfaces. When used with cuprous oxide Chlorothalonil has evidenced a synergism that results in slime control on ship hulls. Dosage levels of 5 to 15% calculated on total formulation are suggested.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Density g/ml (20°C)

Stability

Solubility g/l

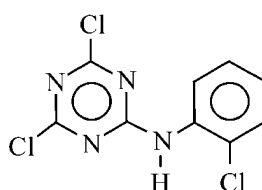
Toxicity data

LD₅₀ oralLD₅₀ percutaneousLC₅₀ on inhalation

Severely irritant to skin, mucosa and eyes.

17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS

17.20. 2,4-Dichloro-6-(2-chloranilino)-1,3,5-triazine



275.53

101-05-3

202-910-5

Anilazine, Dyrene

SIGMA-ALDRICH

odourless, white, crystalline powder

~99

158–162

1.700

sensitive to hydrolysis in alkaline media: at 40°C 1 chloro atom is separated within 3.5 h at pH 10.4, within 1.3 h at pH 11, within 0.7 h at pH 12.5; hydrolytic cleavage of 2 chloro atoms occurs at pH 12.5 within 3.5 h
virtually insoluble in H₂O, soluble in organic solvents

6020 mg/kg mouse

2700 mg/kg rat

400 mg/kg rabbit

>5000 mg/kg rat

>228 mg/m³ (1 h) for rats*Antimicrobial effectiveness/applications*

As is demonstrated by the hydrolysis data, Dyrene is a reactive substance which owes its antimicrobial activity to its reactive chloro atoms enabling the substance to react with nucleophilic cell entities; preferred are those bearing amino and thiol groups. Substituted s-triazines were described as highly fungitoxic by Schuldt & Wolf (1958). Because of being virtually non-phytotoxic Dyrene has become an important fungicide for plant protection. Attempts to introduce Dyrene also for the fungicidal treatment of materials failed more or less, as the activity and the efficacy spectrum could not successfully compete with that of other appropriate fungicides (see Table 136). The antimicrobial treatment of textile material by reacting cotton with Dyrene without affecting fibre properties is reported by Paulus & Pauli (1970); rot-proofing effects can be produced on cotton at substitution levels of around 0.01 only. The cotton cellulose reacts with Dyrene by substitution of one chloro atom of Dyrene.

The cellulose modified of such a kind is resistant to degradation by cellulases. Corresponding finishes are much faster to washing or bleaching and to solvents than those achieved by simple impregnation procedures.

Table 136 Minimum inhibition concentrations (MIC) of Dyrene in nutrient agar

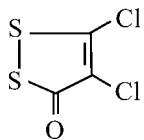
Test organism	MIC (mg/litre)
<i>Alternaria alternata</i>	200
<i>Aspergillus niger</i>	> 1000
<i>Aureobasidium pullulans</i>	200
<i>Chaetomium globosum</i>	200
<i>Cladosporium cladosporioides</i>	100
<i>Lentinus tigrinus</i>	100
<i>Penicillium glaucum</i>	> 1000
<i>Rhizopus nigricans</i>	> 1000
<i>Sclerophoma pityophila</i>	200
<i>Trichoderma viride</i>	> 1000

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.21. Phenylmethanesulphonyl fluoride (PMSF)
Chemical formula	$C_7H_7FO_2S$
Structural formula	$Ph-CH_2-SO_2-F$
Molecular mass	174.20
CAS-No.	329-98-6
EC-No.	206-350-2
EPA TSCA	Section 8 (B) Chemical Inventroy
Synonym/common name	benzylsulphonyl fluoride, α -toluenesulphonyl fluoride
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white powder
Content (%)	~99
Melting point °C	92
Stability	sensitive to hydrolysis under release of volatile hydrogen fluoride; reacts with alcohols and thiols under formation of phenylmethanesulphonyl esters respectively thioesters
Toxicity data	
LD ₅₀ oral	200 mg/kg mouse
LD ₅₀ intraperitoneal	150 mg/kg rat 215 mg/kg mouse
Corrosive to skin, mucous membranes and eyes.	

Antimicrobial effectiveness/applications

PMSF, as a sulphonic acid fluoride very reactive by nature, is known as specific trypsin and chymotrypsin inhibitor (Fahrney and Groid, 1963). Howard et al. (1999) have found that *Pseudomonas chloroaphis* is able to degrade and utilize a polyester-urethane as a sole carbon and energy source using extracellular enzymes. A polyurethane-esterase was isolated; molecular weight: 27000 daltons. PMSF was suited as an effective polyurethane-esterase inhibitor.

Microbicide group (substance class)	17. COMPOUNDS WITH ACTIVATED HALOGEN ATOMS
Chemical name	17.22. 4,5-Dichloro-3H-1,2-dithiol-3-one
Chemical formula	$C_3Cl_2OS_2$

Structural formula	
Molecular mass	187.07
CAS-No.	1192-52-5
EC-No.	214-754-5
Synonym/common name	4,5-dichloro-3-oxo-1,2-dithiole 4,5-dichloro-1,2-dithiolone
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	crystalline lumps tinged with a slight yellowish colour
Content (%)	>90
Melting point °C	52–56
Stability	should be stored at 0–4°C under argon; hydrolyzes in alkaline media
Solubility	soluble in polar organic solvents, emulsifiable in water
Toxicity data	
LD ₅₀ intravenous	13 mg/kg mouse 119 mg/kg rat
Corrosive to skin, mucous membranes and eyes.	

Antimicrobial effectiveness/applications

The chlorinated 1,2-dithiolone is especially effective against slime forming microbe species such as described by Kato & Fukumura (1962); MIC approx. 1 mg/litre nutrient solution. However the very reactive compound is stable and active only in acid media; reduction in activity is observed in neutral media and decomposition under alkaline conditions. The active ingredient may be used for the preparation of slimicides to be applied in pulp and paper mills and in cooling water systems (Clarkson & Clifford, 1991).

18. Surface active agents

Surfactants are characterized by their ability to reduce the surface tension of aqueous fluids; this enables them to act as detergents, wetting agents, emulsifiers. James (1965) has defined them as molecules with two different structural elements, one being a hydrophobic hydrocarbon (water-repellent) group, and the other a hydrophilic polar (water attracting) group. Depending on the charge of the hydrophilic structural element surface active agents are classified as anionic, cationic, amphoteric and non-ionic compounds.

Anionic surfactants exhibit some bactericidal effect only in acid media (pH 2–3), that means in their undissociated state. They present themselves as alkali or amine salts of long-chain fatty acids or alkane sulphonates (e.g. $\text{R-COO}^-\text{Na}^+$, $\text{R-SO}_3^-\text{Na}^+$; $\text{R} = \text{C}_{10}\text{-C}_{12}$ alkyl); in aqueous solution they dissociate to a large anion, responsible for the strong detergent properties and a small cation. The antimicrobial effect of anionic surfactants is restricted mainly to Gram-positive bacteria. Their point of attack is apparently the microbial cell membrane. A survey on the mechanism of the bactericidal action is given by Newton (1960). Acid formulations of anionic surfactants are used as sanitizers in the dairy, beverage and food processing industries, in institutions and homes.

In non-ionic surface active compounds the hydrophilic group usually consists of a chain of ethylene oxide units (ethoxylated compounds). Sorbitan derivatives, such as polysorbates (Tweens) are other examples of non-ionic agents. They do not exhibit significant antimicrobial activity. At low concentrations non-ionic surfactants, and anionic surfactants, too, may potentiate the antimicrobial action of microbicides, such as p-hydroxybenzoates (8.1.11.) and phenol derivatives (7.), by increasing the cellular permeability to the microbicides. At higher concentrations of surface active agents one then observes antagonistic effects caused by the inclusion of active ingredients in the micellar phases of surface active compounds or by complex formation. Micelles are aggregates which spring up in aqueous solutions of surfactants above a certain temperature and a characteristic surfactant concentration CMC (critical micellization concentration). If the CMC is exceeded, the concentrations of the monomers remain constant and the excess surfactant molecules form micelles. At this point which is recognizable by sudden alterations of physical properties of the surfactant solution, e.g. surface tension, osmosis, density, electrical conductivity, starts the inclusion of certain microbicides and their neutralization. Values of

Table 137 Critical micellization concentration and average number of monomers for different surfactants* (Source: Römpp)

Surfactant	Type	CMC (mol/l)	n
$[\text{H}_3\text{C}-(\text{CH}_2)_{11}-\text{N}^{\oplus}(\text{CH}_3)_3]\text{Br}^-$	cationic	14.4	50
$\text{H}_3\text{C}-(\text{CH}_2)_{11}-\text{COO}^-\text{K}^{\oplus}$	anionic	12.5	50
$\text{H}_3\text{C}-(\text{CH}_2)_{11}-(\text{O}-\text{CH}_2-\text{CH}_2)_6-\text{OH}$	non-ionic	0.1	400

* in water at 20°C

CMC and the corresponding average numbers of monomers (n) for different types of surfactants are listed in Table 137.

The neutralization effect of a microbicide bonding to surface active agents above the CMC can be overcome by diluting the mixture to be below the CMC or by increasing the microbicide concentration in the mixture to an amount such that the concentration of free microbicide reaches the effective level. The following relationship is valid and enlightened by Figure 24 (Block, 1983):

$$R = SC + 1$$

R = ratio of total to free (non bound) microbicide concentration

S = surfactant concentration

C = constant, characteristic for each surfactant/microbicide mixture. Anionic and non-ionic surface active compounds are not important as microbicides for the protection of materials. On the contrary, aqueous anionic or non-ionic detergent solutions need the addition of in-tank/in-can preservatives for protection against contamination and proliferation of micro-organisms.

18.1. Quaternary ammonium and phosphonium compounds

18.2. Long-chain alkylamines

18.3. Guanidines and biguanides

18.4. Ampholytes

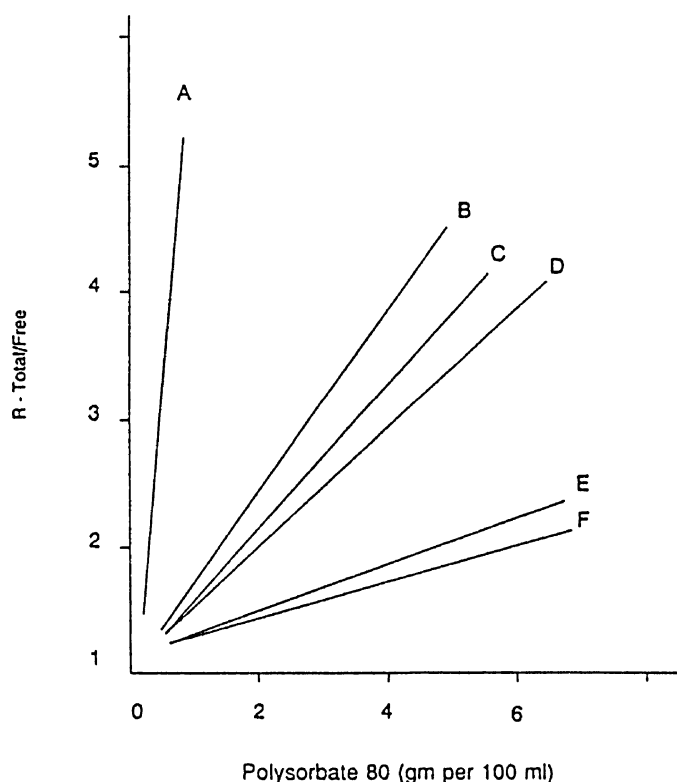
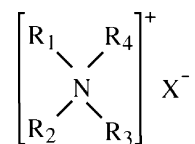


Figure 24 Binding of representative preservatives by a non-ionic surface active agent polysorbate 80, in aqueous solution at 30°C. A = p-Hydroxy-benzoic acid propylester; B = p-Hydroxy-benzoic acid methylester; C = Chlorobutanol; D = Benzoic acid; E = Phenylethyl alcohol; F = Benzyl alcohol.

18.1 Quaternary ammonium and phosphonium compounds

Long-chain quaternary ammonium compounds (QACs) are cationic surface active agents according to the general formula



X is usually chloride or bromide.

The most important QACs may be characterized as follows:

Monoalkyltrimethylammonium salts, e.g. cetyltrimethylammonium bromide (18.1.1.).

Monoalkyldimethylbenzylammonium chloride, e.g. Benzalkonium chloride (18.1.2.). Dialkyldimethylammonium salts, e.g. didecyldimethylammonium halides (18.1.3.). Heteroaromatic ammonium salts (one R stands for a long-chain alkyl group, and the remaining three R are components of an aromatic system such as pyridine, quinoline or isoquinoline) e.g. cetylpyridinium halide and alkylisoquinolinium bromide (18.1.8.).

QACs may be synthesized by alkylation/quaternization of corresponding amines, e.g. fatty amines (18.2.). Instead of the names of many researchers which were involved in the synthesis of QACs and the exploration of their antimicrobial effectiveness, Domagk (1935) is cited, who disclosed the antibacterial activity of the long-chain quaternary ammonium salts. QACs act as algicides, bactericides and fungicides; they are virucidal against lipophilic viruses, but not against hydrophilic viruses; they are not tuberculocidal or sporicidal. Concentrations of 5–10 mg QAC/litre are sufficient to kill Gram-positive bacteria; whereas one needs approximately 50–100 mg QAC/litre to kill Gram-negative bacteria; however, resistant species of *Pseudomonads* are also known. The antimicrobial activity of QACs depends on their structure and size, but especially on the length of the long-chain alkyl group; QACs bearing the C_{14} alkyl group exhibit maximum activity (Cutler et al, 1966). The efficacy of QACs increases with temperature and pH. Alkaline media are most favourable. At $pH < 3$ QACs are widely ineffective. Aralkyl alcohols, especially 3-phenylpropanol (1.8.) potentiate the action of QACs on *Pseudomonas aeruginosa* (Richards & McBride, 1973).

The QACs' mode of action is rather complex. Of course they belong to the membrane-active microbicides which damage the cytoplasmic membrane controlling the cell permeability (Hugo, 1965). QACs can be characterized as microbicides of low toxicity which with normal precautions may be handled safely especially with regard to the very low use dilutions. They find many and varied uses. However, their usefulness in practice is severely limited by the fact that they can cause foam problems and are incompatible with a wide variety of compounds, especially anionic surfactants, organic matter, including milk, serum and faeces. If one pays attention to these properties of the QACs, they can successfully be used as active ingredients in disinfectants for application in hospitals, households and the food and beverage industry; QACs are used as microbicides in the sugar refining industry; they are added to process water to inhibit the proliferation of slime forming organisms and algae. 5–20 mg QAC/l injection water or brine used in secondary oil recovery prevent the growth of microorganisms which cause the plugging of the subterranean sand formations thus stopping the operation. In outdoor swimming pools QACs may act as powerful algicides. Algae and lichens on stone surfaces are killed by a wash with an aqueous solution containing 0.5–1% QAC. In combination with other fungicides QACs are applied for the temporary protection of freshly cut and sawn timber against stain and mould fungi.

Due to the positively charged structural elements of the quaternary molecule it has an affinity to negatively charged fabric. The antimicrobial treatment of textile material with QACs takes advantage of this phenomenon. The substantivity of QACs is extraordinarily strong to cotton which has gained anionic properties by chemical modification (Paulus, 1971).

Another possibility for surface-bonded antimicrobial activity is the use of organosilicon quaternary ammonium salts (Si-QAC). Isquith et al. (1972) have demonstrated that the hydrolysis product of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (18.1.10.) exhibits antimicrobial activity against a broad range of micro-organisms while chemically bonded on a variety of surfaces.

The microbicidal activity of quaternary surface active compounds is not restricted to ammonium salts; longchain quaternary phosphonium salts (18.1.11.) are similar in effectiveness and properties.

The series of QACs described in this section does not claim to be complete. As can be seen from the general formula cited above it is apparent that a wide scope exists for synthesis of antimicrobial QACs; in consequence numerous QACs are offered for practical application as microbicides. The QACs listed in the following may be regarded as prototypes illustrating the wide variety of modifications which are possible within this class of microbicides.

Microbicide group (substance class)	18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS
Chemical name	18.1.1. Hexadecyltrimethylammonium bromide
Chemical formula	$C_{19}H_{42}BrN$
Structural formula	$\left[\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-(\text{CH}_2)_{15}-\text{N}-\text{CH}_3 \\ \\ \text{CH}_3 \end{array} \right]^+ \text{Br}^-$
Molecular mass	364.46
CAS-No.	57-09-0
EC-No.	200-311-3; EEC-no. 44
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	Cetyltrimethylammonium bromide (CTAB), Cetrimonium bromide
Supplier	HENKEL, HOECHST, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	almost colourless, odourless crystals
Content (%)	min. 99
Melting point °C	248–251
Flash point °C	244
pH (1% in H ₂ O)	5–7
Ionicity	cationic
Stability	stable in acid solutions; incompatible with anionic detergents, peptides, zinc salts, polymeric phosphates, pectins, strong oxidizing and reducing agents
Solubility	soluble in H ₂ O (promotes foam); highly soluble in lower alcohols, poorly soluble in acetone, virtually insoluble in non-polar solvents
Toxicity data	
LD ₅₀ oral	410 mg/kg rat
LD ₅₀ intravenous	44 mg/kg rat
	32 mg/kg mouse
LD ₅₀ intraperitoneal	106 mg/kg mouse
	125 mg/kg rabbit
LD ₅₀ subcutaneous	125 mg/kg rabbit
	100 mg/kg guinea pig
Irritant to skin and mucous membranes. Severely irritant to the eyes.	
Ames test negative.	

Antimicrobial effectiveness/applications

The antimicrobial efficacy and CTAB's the spectrum of activity is demonstrated by the microbicidal concentration for different species of bacteria, yeasts and fungi in Table 138 (Wallhäusser, 1984).

Apparently CTAB is highly effective against Gram-positive bacteria, but by far not that active against Gram-negative bacteria and fungi. The complex Gram-negative cell wall is in comparison to the Gram-positive cell membrane the more solid barrier for CTAB. Concentration dependent effects of CTAB on the structure of cell walls and the permeation of cell membranes especially those of Pseudomonads were examined by Wölfel et al. (1985). The survival of *Pseudomonas aeruginosa* cells even in the presence of concentrations of CTAB as high as 160 mg/litre is explained by the formation of cell aggregates which is induced by the surface active compound and protect the cells within the conglomerates. CTAB is mainly used as an active ingredient in disinfectants. As a preservative permitted for use in cosmetics it is listed in the corresponding EC positive list with a maximum authorized concentration of 0.1%. Optimum pH range: 4–10. Percentage of use in US cosmetics formulations: 0.03%.

Table 138 Minimum microbicidal concentrations of CTAB (MMC) after 24 h at 22°C

Test organism	MMC (mg/litre)
<i>Staphylococcus aureus</i>	1.5
<i>Streptococcus lactis</i>	0.6
<i>Bacillus subtilis</i>	6
<i>Escherichia coli</i>	25
<i>Pseudomonas aeruginosa</i>	> 50
<i>Serratia marcescens</i>	25
<i>Desulfovibrio desulfuricans</i>	50
<i>Mycobacterium 607</i>	6
<i>Streptomyces griseus</i>	25
<i>Candida albicans</i>	25
<i>Penicillium chrysogenum</i>	> 50

Microbicide group (substance class)

18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

Chemical name

18.1.2. N-Alkyl(C₈-C₁₈)-N,N-dimethyl-N-benzylammonium chloride = Benzalkonium chloride

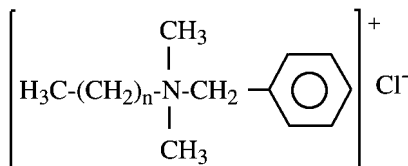
The number of carbon atoms in the alkyl group may differ between n-C₈ and n-C₁₈ and Benzalkonium chloride may contain several different corresponding alkyl groups. Examples for carbon chain distribution in Benzalkonium chloride:

C ₁₂ 3%, C ₁₄ 95%, C ₁₆ 2%	CAS-no. 68424-85-1	EC-no. 270-325-2
C ₁₂ 70%, C ₁₄ 26%, C ₁₆ 4%	CAS-no. 68424-85-1	EC-no. 269-919-4
C ₁₂ 40%, C ₁₄ 50%, C ₁₆ 10%	CAS-no. 68424-85-1	EC-no. 270-325-2
C ₁₀ 2%, C ₁₂ 57%, C ₁₄ 23%, C ₁₆ 11%, C ₁₈ 7%	CAS-no. 61789-71-7	EC-no. 263-080-8
C ₁₂ 70%, C ₁₄ 30	CAS-no. 68391-01-5	EC-no. 264-151-6

The antimicrobial efficacy of the compound increases with the portion of C₁₄-alkyl.

Supplier: BAYER, BUCKMANN, HOECHST, LONZA, SIGMA-ALDRICH, THOR and others.

Structural formula



Average molecular mass

408

CAS-No.

68424-85-1

EC-No.

270-325-2; EEC-no. 16 in Appendix B

EPA TSCA

Inventory

Synonym/common name

Benzalkonium chloride

Supplier

LONZA

Chemical and physical properties

Appearance

white-yellowish power with an aromatic odour

Content (%)

min. 99

Melting point °C

61

Bulk density g/l

350

Flash point °C

>100

Ignition temperature °C

>365 (deposited dust)

pH (1% aqueous solution)

5–8 at 20°C

Ionicity

cationic

Stability

stable at normal conditions; compatible with non-ionic surfactants; not compatible with anionics; stable at pH 1–12

Solubility g/l (20°C)

250 in H₂O, soluble in lower alcohols and ketones

Toxicity data (source: LONZA)

LD₅₀ oral 600 mg/kg rat

Irritant to skin and mucosa; causes severe eye irritation.

Ecotoxicity:

Toxicity to fish

LC₅₀ for Rainbow trout 0.93 mg/l (96 h)

for Bluegill sunfish 0.515 mg/l (96 h)

NOEC for Fathead minnow 0.032 mg/l (34 d)

Toxicity to daphnia

EC₅₀ for Daphnia magna 0.0058 mg/l (48 h) – immobilization

NOEL (reproduction test) 0.0042 mg/l (21 d)

Toxicity to activated sludge organisms

EC₅₀ (respiration inhibition) 10 mg/l (OECD method 209)

Biodegradation (OECD method 301 B)

84% within 28 days (readily biodegradable)

Antimicrobial effectiveness/applications

Benzalkonium chloride is especially effective between pH 6 and 8. Its broad spectrum of activity covers bacteria, yeasts, fungi, algae, lichens and slime-forming organisms. Diluted solutions of Benzalkonium chloride are therefore ideal for eliminating fungi, algae and lichens for example from finished coatings, plaster and stone, concrete and wooden surfaces. As a surface active compound it greatly reduces the surface tension of water; good wetting and penetration depth therefore are guaranteed when using 0.75–1.5% dilutions of Benzalkonium chloride as a substrate pre-treatment agent.

Benzalkonium chloride is also used as an active ingredient in disinfectants. With regard to this application one has carefully to observe the relatively weak activity of Benzalkonium chloride against Pseudomonads and its inactivation by anionic detergents and organic matter. In the food industry the active ingredient profits from being easily rinsed off and being tasteless and odourless.

Because of its distinctive algicidal and slimicidal effects Benzalkonium chloride formulations may be used for the treatment of cooling and swimming pool water. However, the foam promoting effect of Benzalkonium chloride even at low concentrations (20–40 mg/l) sets limits to this application. Recommended addition rates are 2–10 mg/l.

Very limited is also the use of Benzalkonium chloride as a preservative for the in-can/in-tank protection of aqueous functional fluids (including cosmetics) because of its cationic character. However, it is listed as a preservative on the EEC positive list for cosmetics.

Microbicide group

(substance class)

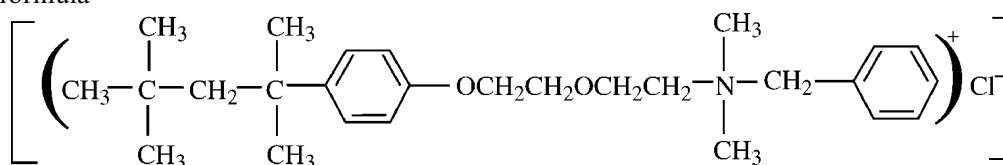
Chemical name

Chemical formula

Structural formula

18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

18.1.3. Diisobutylphenoxyethoxyethyl-dimethylbenzylammonium chloride

C₂₇H₄₂ClNO₂

Molecular mass

448.10

CAS-No.

121-54-0

EC-No.

204-479-9; [NO49]A, EEC-no.: not applicable

EPA Reg.

approval for antimicrobial application

EPA TSCATS

Data base, Jan. 2001

Synonym/common name	benzyldimethyl-p-(1,1,3,3-tetramethylbutyl) phenoxyethoxy-ethylammonium chloride, Benzethonium chloride
Supplier	LONZA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to off white, odourless powder
Content (%)	>97
Melting point °C	165
Bulk density g/ml (20°C)	0.44
Ignition temperature °C	approx. 380
PH (1% in H ₂ O)	5–6.5
Ionicity	cationic
Stability	stable to melting point and in media of pH 1 to 12; precipitation and inactivation by anionic compounds and proteins
Solubility	highly soluble in water, producing a foamy, soapy solution; soluble in lower alcohols, glycols and acetone
Toxicity data	
LD ₅₀ oral	368 mg/kg rat 338 mg/kg mouse
LD ₅₀ intraperitoneal	16.5 mg/kg rat 7.8 mg/kg mouse
LD ₅₀ intravenous	19 mg/kg rat 30 mg/kg mouse
LD ₅₀ subcutaneous	119 mg/kg rat
Mutagenicity: Ames test negative.	
Irritant to skin and mucosa. In tests with rabbits 30 µg caused severe eye irritation.	
Ecotoxicity (source: LONZA):	
LC ₅₀ for Bluegill sunfish	< 1 mg/l (96 h)
for Fathead minnow	< 1 mg/l (96 h)
Biological degradation (30 days):	40.0%

Antimicrobial effectiveness/applications

Benzethonium chloride exhibits antimicrobial efficacy against bacteria, yeasts and fungi. However, as is characteristic for such QAC's, it is not reliable in effectiveness against Pseudomonads.

Use areas:

Hospital area disinfection. – Active ingredient in disinfectants for instruments and such to be used in the veterinary field. Preservative for cosmetics not coming into contact with mucous membranes. Percentage of use in US cosmetic formulations: 0.11%.

Microbicide group (substance class)	18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS
Chemical name	18.1.4. Di-n-decyl-dimethylammonium chloride (DDAC)
Chemical formula	C ₂₂ H ₄₈ NCl
Structural formula	$\left[\begin{array}{c} \text{H}_3\text{C} \quad (\text{CH}_2)_9\text{-CH}_3 \\ \quad \diagdown \quad \diagup \\ \text{N} \\ \quad \diagup \quad \diagdown \\ \text{H}_3\text{C} \quad (\text{CH}_2)_9\text{-CH}_3 \end{array} \right]^+ \text{Cl}^-$
Molecular mass	362.09
CAS-No.	7173-51-5

EC-No.	230-525-2
EPA-Reg.	approval for antimicrobial application; TSCA Inventory
Synonym/common name	N,N-didecyl-N,N-dimethylammonium chloride, Didecyldimonium chloride
Supplier	LONZA, THOR
Chemical and physical properties	
Appearance	clear, colourless to pale yellow liquid with an isopropanol-like odour
Content (%)	~50 (isopropanol ~20; H ₂ O ~30)
Boiling point/range °C (101 kPa)	>80
Stock point °C	<-30
Density g/ml (20°C)	0.89
Vapour pressure hPa (20°C)	7.5×10^{-6} (100% a. i.)
Viscosity mPa·s (20°C)	25
Surface tension mN/m (20°C)	27 (1% aqueous solution)
Refractive index nD (20°C)	1.417–1.422
Flash point °C	29
Ionicity	cationic
pH (1% in H ₂ O)	6.5–8
Stability	stable over the pH range 2–10 and up to 120°C; not compatible with anionic compounds (precipitation/inactivation); photolytically stable
Solubility	miscible in any ratio with water and lower alcohols
Toxicity data	
LD ₅₀ oral	645 mg/kg rat
LD ₅₀ dermal	2600 mg/kg rat
No sensitization according to the Magnusson – Kligman test.	
Strongly irritant to skin mucosa and eyes. Not mutagenic according to different in-vivo and in-vitro tests; not teratogenic in rats.	
Ecotoxicity (source: LONZA):	
LC ₅₀ for Rainbow trout	2 mg/l (96 h)
EC ₅₀ for Daphnia magna	<1 mg/l (48 h)
ErC ₅₀ for algae (Scenedesmus sp.)	0.33 mg/l (72 h)
EC ₁₀ for bacteria (Pseudomonas sp.)	0.13 mg/l (16 h)
Biodegradability: 87–94 (“biologically well degradable”); test period: 28 d; method: OECD 302 B.	
A comprehensive review of the environmental chemistry, fate, behavior and aquatic toxicity of DDAC was conducted by Juergensen et al (2000).	

Antimicrobial effectiveness/applications

DDAC exhibits microbicidal activity against a wide range of bacteria, fungi and yeast. The algaestatic concentration of the 50% DDAC formulation amounts to 0.5 mg/l; algaecidal efficacy is achieved by 1.0 mg/l. The virucidal effectiveness of DDAC is limited to enveloped viruses.

When compared to other QACs didecyl-dimethylammonium chloride maintains an unusually high level of activity in the presence of organic matter, proteinaceous soil and hard water. This is confirmed by the fact that 400 mg a. i./ml are effective in the presence of 5% blood serum against *Staphylococcus aureus* according to the AOAC Use-Dilution Test. The compound is able to maintain its bactericidal activity even in the presence of residual amounts of anionics which normally are detrimental to the performance of QACs. Because of its favourable performance characteristics didecyl-dimethylammonium chloride is used in a great variety of application areas: as active ingredient in disinfectants, sanitizers, and cleaners for use in hospitals, homes, institutions, dairy, farm, and industrial areas; as a water treatment microbicide for cooling towers and secondary oil recovery; as a microbicide for the protection of textile material against permanent staining by the attack of mould-producing fungi and as an active ingredient in solutions for the temporary protection of freshly sawn timber against the growth of wood discolouring fungi. DDAC is also used as a molluscicide.

Microbicide group (substance class)	18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS
Chemical name	18.1.5. Dioctyl-dimethylammonium chloride
Chemical formula	$C_{18}H_{40}ClN$
Structural formula	$\left[\begin{array}{c} H_3C \quad (CH_2)_7-CH_3 \\ \diagdown \quad / \\ N \\ / \quad \diagdown \\ H_3C \quad (CH_2)_7-CH_3 \end{array} \right]^+ Cl^-$
Molecular mass	305.98
CAS-No.	5538-94-3
EC-No.	226-901-0
EPA TSCA	Inventory list
Synonym/common name	N, N-dioctyl-N,N-dimethylammonium chloride
Supplier	LONZA
Chemical and physical properties	
Appearance	clear, colourless to yellowish liquid with an ethanol-like odour
Content (%)	50–52 (H ₂ O: 36–40; ethanol: min. 8)
Stock point °C	<5
Density g/ml (20°C)	0.9320
Viscosity mPa·s (20°C)	22
Surface tension mN/m (20°C)	32 (1% aqueous solution)
Flash point °C	50
Ionicity	cationic
pH (1% in H ₂ O)	6.5–8
Stability	not compatible with anionic compounds (e.g. detergents); promotes considerably less foam in aqueous media than other QAC's
Solubility	completely miscible with H ₂ O; highly soluble in lower alcohols
Toxicity data (source: LONZA)	
LD ₅₀ oral	1025 mg/kg rat
In tests with rabbits (method: DOT) corrosive to skin (exposure: 24 h), severely irritant to the eyes.	
Ecotoxicity:	
LC ₅₀ for Rainbow trout	<1 mg/l (96 h)
for Bluegill sunfish	1 mg/l (96 h)
Biodegradation: approx. 90% in 10 days – readily degradable.	

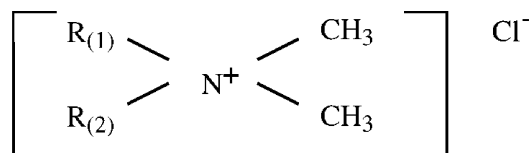
Antimicrobial effectiveness/applications

The antimicrobial activity of dioctyl-dimethylammonium chloride corresponds approximately to that of other QAC's. The algacidal concentration of the 50% formulation was shown to be 1–5 mg/l.

Use areas are disinfectants and disinfectant cleaners, agriculture applications, food and dairy industry. As foam promotion by active concentrations is not significant the microbicide is preferably used as an algacide and slimicide for water treatment (swimming pools, cooling towers, paper industry, etc.).

Microbicide group (substance class)	18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS
Chemical name	18.1.6. N-Decyl-N-isononyl-N,N-dimethylammonium chloride
Chemical formula	$C_{21}H_{46}ClN$

Structural formula

R₁ = Decyl (C₁₀H₂₁) / R₂ = isononyl (C₉H₁₉)

Molecular mass

348.06

CAS-No.

138698-36-9

EC-No.

270-331-5

Supplier

LONZA

Chemical and physical properties

Appearance

clear, slightly yellow liquid with a mild aromatic odour

Content (%)

70–72 (propylene glycol 10–12; H₂O 11–20)

Stock point °C

–5

Density g/ml (25°C)

0.93

Viscosity mPa·s (25°C)

<150

Surface tension mN/m (20°C)

33 (1% aqueous solution)

Flash point °C

>93

pH (10% in H₂O)

6.5–9

Ionicity

cationic

Stability

stable under normal conditions; not compatible with anionics

Solubility g/l

highly soluble in water and lower alcohols

Toxicity data (source: LONZA)

LD₅₀ oral

320 mg/kg rat

Corrosive to the rabbit skin (exposure period 4 h).

Mutagenicity: Ames-test negative.

Ecotoxicity:

LC₅₀ for Rainbow trout

2 mg/l (96 h)

for Bluegill sunfish

1–2 mg/l (96 h)

EC₅₀ for Daphnia magna

<1 mg/l (48 h)

Biodegradation: approx. 90% within 10 days – readily biodegradable.

Antimicrobial effectiveness/applications (source: LONZA)

The bactericidal efficacy has been tested and shown according to different test procedures:

DGHM (Germany) – AOAC (USA) – AFNOR (France).

The QAC exhibits algacidal actions in a concentration of 30 mg/l against *Chlorella pyrenoidosa*, of 7.5 mg/l against *Phormidium innundatum*, of 30 mg/l against Arizona mustard.

Use areas:

Disinfectant and disinfectant cleaners. Agriculture applications, food and dairy industry.

Water treatment (swimming pools, cooling towers, etc.).

Disinfection of surgical instruments in washing machines.

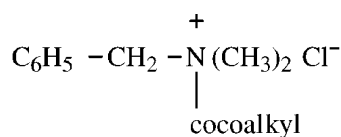
Microbicide group (substance class)

18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

Chemical name

18.1.7. Benzyl-cocoalkyl-dimethylammonium chlorides

Structural formula

cocoalkyl : mainly lauryl (C₁₂H₂₅), myristyl (C₁₄H₂₉), palmityl (C₁₆H₃₃)

CAS-No. 61789-71-7
 EC-No. 263-080-8
 EPA TSCA Inventory list
 Supplier LONZA

Chemical and physical properties

Appearance colourless liquid with a characteristic odour
 Content (%) 50 (in H₂O)
 Boiling point/range °C (101 kPa) approx. 100
 Melting point °C approx. -5
 Density g/ml (20°C) 0.978-0.988
 Viscosity mPa·s (20°C) 100-300
 Flash point °C >100
 Ionicity cationic
 Stability stable at normal conditions; not compatible with anionics
 Solubility completely miscible with water

Toxicity data (source: LONZA)

LD₅₀ oral 500-2000 mg/kg rat

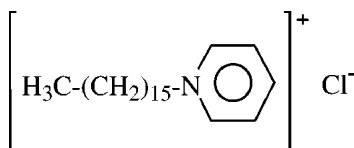
In tests with rabbits corrosive to skin.

The ecotoxicity of the active ingredient corresponds to 18.1.2. This statement is also valid for the antimicrobial effectiveness and use areas.

Microbicide group (substance class)

18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

Chemical name 18.1.8. 1-Hexadecylpyridinium chloride monohydrate
 Chemical formula C₂₁H₃₈ClN·H₂O
 Structural formula



Molecular mass 358.01
 CAS-No. 6004-24-06
 EC-No. 204-593-9
 Synonym/common name Cetylpyridinium chloride
 Supplier RIEDEL DE HAEN, SIGMA-ALDRICH

Chemical and physical properties

Appearance white crystalline powder with a characteristic odour
 Content (%) min. 98
 Melting point °C 79-82
 Ionicity cationic
 PH (1% aqueous solution) 6-7
 Stability not compatible with anionic compounds, proteins, zinc salts and polyphosphates
 Solubility highly soluble in water and alcohols

Toxicity data

LD₅₀ oral 200 mg/kg rat
 400 mg/kg rabbit
 LD₅₀ intraperitoneal 10 mg/kg mouse
 25 mg/kg rabbit
 LD₅₀ intravenous 30 mg/kg rat
 35 mg/kg rabbit
 LD₅₀ subcutaneous 300 mg/kg rabbit

Irritant to skin and mucosa. A 1:5000 aqueous solution caused light to no eye irritation in tests with rabbits.

Antimicrobial effectiveness/applications

Cetylpyridinium chloride acts highly bactericidal: 12 mg/l kill Staphylococci, but 170 mg/l are required to kill Pseudomonads. The microbicide is mainly used as an active ingredient in antiseptics, e.g. in deodorants, and disinfectants.

Microbicide group (substance class)

Chemical name

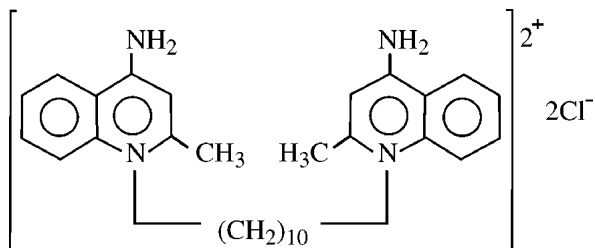
Chemical formula

Structural formula

18. 1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

18.1.9. Dequalinium chloride

$C_{30}H_{40}Cl_2N_4$



Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

527.58

522-51-0

208-330-9

1, 1'-decamethylenebis-(4-amino-chinaldinium)-dichloride

FLUKA

Chemical and physical properties

Appearance

Content(%)

Melting point °C

Ionicity

Stability

white to yellow crystalline powder

~95

>300

cationic

not compatible with anionic compounds, such as phenol derivatives,

detergents and other anionic surface active agents

soluble in H_2O and lower alcohols

Solubility

Toxicity data

LD_{50} intraperitoneal

LD_{50} intravenous

LD_{50} subcutaneous

Irritant to skin and mucosa.

18 mg/kg mouse

19 mg/kg mouse

70 mg/kg mouse

Antimicrobial effectiveness/applications

Effective against fungal and bacterial infections, however, has not gained major importance as an active ingredient in disinfectants.

Microbicide group (substance class)

Chemical name

Chemical formula

18.1. QUATERNARY AMMONIUM AND PHOSPHONIUM COMPOUNDS

18.1.10. 3-(Trimethoxysilyl)-propyl-dimethyloctadecylammonium chloride

$C_{26}H_{58}ClNO_3Si$

Structural formula	$[(\text{H}_3\text{C-O})_3\text{Si-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}(\text{CH}_3)_2]^+\text{Cl}^-$ $\text{C}_{18}\text{H}_{37}$
Molecular mass	496.30
CAS-No.	27668-52-6
EC-No.	248-595-8
EPA Reg.	approval for antimicrobial application
EPA TSCA	Section 8 (B) Chemical Inventory
Supplier	DOW CORNING, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	clear, to pale yellow liquid with an alcoholic odour
Content (%)	approx. 50 (in methanol)
Density g/ml (20°C)	0.883
Flash point °C	11
pH (10% in H ₂ O)	approx. 5
Stability	stable under normal conditions; inactivation by anionic surfactants; hydrolyzes in aqueous media to 3-(trihydroxysilyl)-propyl-dimethyl-octadecylammonium chloride which is regarded as the correct active ingredient, as it is capable of binding to a variety of natural and synthetic substrates to produce a durable surface or fabric coating.
Solubility	soluble in water and alcohols

Toxicity data (source: BIOSHIELD)

Irritant to skin, mucosa and eyes. The material does not contain carcinogens, or teratogens, or reproductive toxins, or sensitizers.

Disposals can be de-activated by the addition of an anionic surfactant.

Antimicrobial effectiveness/applications

The hydrolysis product of the $(\text{H}_3\text{C-O})_3\text{Si-QAC}$ exhibits bacteriostatic, fungistatic and algaestatic activity, if it is chemically bonded by a condensation reaction to surfaces containing corresponding reactive functional groups, such as woven and non-woven textiles, polymers and coatings, leather, wood. The condensation reaction proceeds according to the scheme which is shown in Figure 25.

The chemical is not removed from treated materials by repeated washing with water and its antimicrobial activity could not attributed to a slow release of the chemical or of octadecyl-propyl-dimethylammonium chloride. Isquith et al. (1972) used ^{14}C -Si-QAC-treated cellulose acetate sheet to gain evidence of substantial antimicrobial activity of Si-QAC. The fact that the efficacy of Si-QAC is not dependent on the slow release of the QAC lends support to the theory that the QAC acts on the membrane or cell wall but probably not on intracellular organelles.

It is recommended to apply 0.1–1.0% active ingredient to give an effective final treatment.

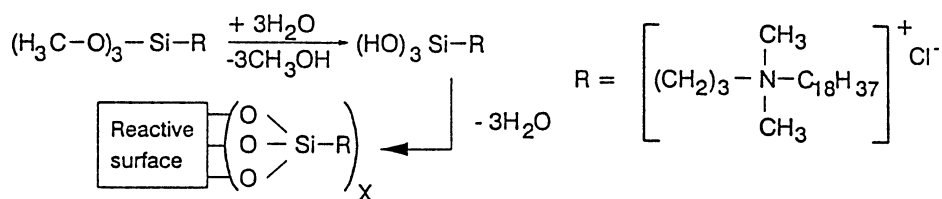


Figure 25 Hydrolysis and condensation of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride.

18.1.11. Polymeric quaternary ammonium compounds. Polymeric quaternary ammonium salts are the result of reactions between nucleophilic monoamines or diamines and electrophilic di-halo compounds (Renbaum, 1973). They can be further cross-linked with ammonia, ethylenediamine or other reactive amines. Generally the polymeric QACs are not isolated but produced as viscous solutions containing 50–60% active ingredient. The molecular weights of linear polymeric QACs vary between 3000 and 10 000 and 30 000 and 50 000 for cross-linked compounds.

It has been found that the polymeric QACs have properties which make them uniquely different from the microbicidal QACs described hereto. They do not produce foam, even at relatively high concentrations in water and reportedly they are of low toxicity. As ordinary microbicidal QACs they are highly effective against algae and slime forming micro-organisms and therefore used as algicides and slimicides for water treatment. But according to results presented by Hollis et al. (1991) commercial solutions of polymeric QACs are also capable of preserving aqueous functional fluids such as starch slurries and metal working fluids. Another possible application for polymeric QACs is the control of fouling by marine and fresh water molluscs, particularly the control of fresh water Asiatic clams of genus *Corbicula*, the most common of which is *C. fluminea* (Hollis & Lutey, 1988). Polymeric QACs can control the population of adult *C. fluminea* and inhibit the attachment of juveniles at concentrations of 2–8 mg/litre.

Examples of polymeric QACs:

Poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride]

Poly[hydroxyethylene(dimethyliminio)ethylene(dimethyliminio)methylene dichloride]

Poly[hydroxyethylene(dimethyliminio)-2-hydroxypropylene(dimethyliminio)-methylene dichloride]

[N-[3-(dimethylammonio)propyl]-N'[3-(ethyleneoxyethylenedimethylammonio)propyl]urea dichloride]

α -4-[1-tris(2-hydroxyethyl)ammonium chloride-2-butenyl]poly[1-dimethyl-ammonium chloride-2-butenyl]- ω -tris(2-hydroxyethyl) ammonium chloride

One example will be described in more detail showing the characteristics which are valid also for other polymeric QACs.

Microbicide group (substance class)	18.1.11. POLYMERIC QUATERNARY AMMONIUM COMPOUNDS
Chemical name	18.1.11.1. N,N-Dimethyl-2-hydroxypropyl-ammonium chloride polymer
Chemical formula	$[\text{C}_5\text{H}_{12}\text{NO}]^+_n \text{Cl}^-$
Average molecular mass	20 000
CAS-No.	25988-97-0
EC-No.	not applicable (notified in the EINECS inventory: N 269; conditionally acceptable)
EPA TSCA	Inventory
Supplier	BUCKMAN, LONZA
Chemical and physical properties	
Appearance	colourless to slightly yellow liquid with a faint odour of ammonia
Content (%)	59–61 (in H_2O)
Boiling point/range °C (101 kPa)	>100
Solidification point °C	<–15
Density g/ml (20°C)	1.170
Viscosity mPa·s (23°C)	1900
Surface tension mN/m (20°C)	56 (1% aqueous solution)
Flash point °C	> 100
Ionicity	Cationic
pH (1% in H_2O)	7.7–8.7
Stability	stable at normal conditions; not compatible with anionics; non foaming
Solubility	highly soluble (miscible) in water
Toxicity data (source: LONZA)	
LD ₅₀ oral	>2000 mg/kg rat
In tests with rabbits no skin irritation (OECD method 404; exposure time: 4 h); mildly irritant to the eyes (OECD method 405).	
Ecotoxicity:	
LC ₅₀ for Zebra fish	0.27 mg/l (96 h)
E _r C ₅₀ for algae (<i>Scenedesmus</i> sp.)	0.18 mg/l (72 h), growth inhibition

EC₅₀ for activated sludge organisms 150 mg/l (3 h), respiration inhibition
 Biodegradability: 81% within 28 days (OECD method 301 B); biologically well degradable.

Antimicrobial effectiveness/applications

Because of its high activity against algae, and slime forming micro-organisms including fungi, the polymeric QAC is used as a water treatment microbicide at concentrations between 5 and 20 mg/litre which are effective without causing foam problems. However, one has to give regard to the fact that similar to ordinary QACs the polymeric QACs, too, are easily adsorbed by organic material. It is therefore recommended to pre-clean the water system to be treated or to reduce the content of adsorbing materials in the system.

Microbicide group (substance class)

18.1.11. POLYMERIC QUATERNARY AMMONIUM COMPOUNDS

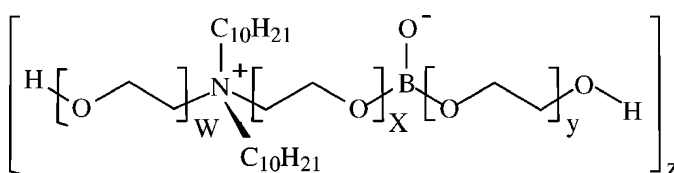
Chemical name

18.1.11.2. Oligomeric {[Didecyl(2-hydroxyethyl-poly-2-oxidoethyl)-(poly-2-oxidoethyl)-(ω-hydroxy-poly-ethoxy)ammonium.O, O]⁻borate}

Chemical formula

variable

Structural formula



Molecular mass

approx. 1850

CAS-No.

214710-34-6

EC-Notification-No.

N 074

Synonym/common name

didecylpolyoxyethyl ammonium borate (DPAB),
alkylpolyethoxy ammonium borate

Supplier

RUETGERS ORGANICS

Chemical and physical properties

Appearance

light coloured liquid of medium viscosity

Content (%)

57–61

Boiling point/range °C(101 kPa)

not detectable (rapid decomposition above) 120°C)

Density g/ml (20°C)

0.96–1.01

Viscosity mPa·s (20°C)

139–180

Surface tension mN/m (20°C)

25.9 (0.1% in H₂O)

pH value

9–11

Stability

stable in aqueous solutions between pH 7.5 and 11.5; optimum stability between pH 9–10; rapid hydrolysis at pH < 3; sensitive to heat (>80°C); light stable; highly surface active (promotes foaming); high concentrations of electrolytes (e.g. NaCl) can cause precipitation.

Solubility

soluble and miscible in water at all concentrations; highly soluble in polar solvents (e.g. alcohols, glycols etc.).

Toxicity data (source: RUETGERS ORGANICS)

LD₅₀ oral

>2000 mg a.i./kg male rat
500–2000 a.i./kg female rat

Dermal exposure: uptake of a.i. is not significant. Irritant to skin and mucosa; not a skin sensitizer. Not mutagenic according to Ames test, cytogenicity study and gene mutation assay. Result of a 90-day-dietary toxicity study in rat: NOEL 50 mg/kg/day.

Ecotoxicity:

LC₅₀ for fish (*brachydanio rerio*) 0.5–1 mg/l (96 h)

EC₅₀ for *Daphnia magna* 0.76 mg/l (48 h)

DPAB is highly immobile in the environment, as it is readily absorbed in soil. It is degradable in soil and accordingly classified as 'inherently biodegradable'.

Antimicrobial effectiveness/applications

DPAB is active against many Gram-positive and Gram-negative bacteria as well as against fungi and yeasts (see Table 139). Due to its high activity against basidiomycetes and other wood-destroying fungi, as well as against insects DPAB is preferably used in wood preservatives. High penetration with fast fixation guarantees long lasting protection.

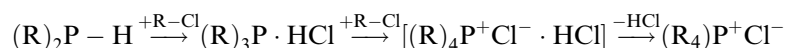
As possible applications are indicated use in cleaning and sanitary agents for household, antimicrobial treatment of textiles and water. Suggested concentration: 100–200 mg/l of the formulation.

In contrary to QAC's which contain chloride ions DPAB does not increase corrosion of metals in aqueous media but acts as an corrosion inhibitor.

Table 139 Minimum inhibition concentrations (MIC) of DPAB (57–61%). (Source: RUETGERS ORGANICS)

	MIC in mg/l (ppm)
Bacteria	
<i>Escherichia coli</i>	500
<i>Micrococcus flavus</i>	140
<i>Staphylococcus epidermis</i>	90
<i>Proteus vulgaris</i>	90
<i>Salmonella typhimurium</i>	85
<i>Staphylococcus aureus</i>	80
<i>Enterobacter cloacae</i>	80
<i>Pseudomonas aeruginosa</i>	55
<i>Shigella sonnei</i>	50
<i>Klebsiella pneumodia</i>	40
Yeasts	
<i>Saccharomyces cerevisiae</i>	85
<i>Candida albicans</i>	50

18.1.12. *Tetraalkylphosphonium compounds (TAPC's)*. TAPC's are obtained by alkylation of secondary or tertiary phosphines with alkylhalides at temperatures of 80–100°C. Intermediarily originates a tetraalkylphosphonium halide-hydrohalide complex which, if exposed to vacuum at the elevated temperatures, releases hydrohalide and is transformed to tetraalkylphosphonium halide:



Long-chain TAPC's are surface active agents which are more heat resistant than QAC's. Uexküll (1976) reports of TAPC's which are highly effective against bacteria – unexpectedly against *Pseudomonads*, too –, fungi, yeasts, algae and slime forming organisms. Remarkable is that these microbicides in hard water are not inhibited in their antimicrobial activity. The long-chain TAPC's are incompatible with anionics, non-ionics, proteins, and cationic compounds also can adversely affect their efficacy.

Di-n-C₉ to di-n-C₁₁ alkyl phosphonium halides are the most effective ones, especially with regard to their activity against *Pseudomonads*.

Compounds with C₁₆ or longer alkyl chains are still highly effective, however, their special merit is their low oral toxicity.

Examples

1. A = Didecyl-dimethylphosphonium chloride, $(C_{10}H_{21})_2P^+(CH_3)_2Cl^-$, colourless mass, mp 172–176°C.
2. B = Didodecyl-ethyl-isobutylphosphonium chloride, $(C_{12}H_{25})_2P^+(C_2H_5)(H_2C-CH_2(CH_3)_2)Cl^-$, colourless mass, mp 53–56°C.
3. C = Dihexyldecyl-ethyl-isobutylphosphonium chloride, $(C_{16}H_{33})_2P^+(C_2H_5)H_2C-CH_2(CH_3)_2Cl^-$, colourless mass, mp 60–67°C

Table 140 Minimum inhibition concentrations (MIC in mg/l) of tetraalkylphosphonium chlorides (Source: Uexküll, 1976)

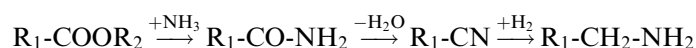
TAPC	Test organisms			
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Aspergillus niger</i>	<i>Chlorella vulgaris</i>
A	16	0.5	0.25	0.125
B	16	0.5	1.0	0.5
C	16	0.5	0.5	0.5

TAPC's can be provided for practical applications as concentrates (e.g. 50%) in water which are completely miscible with water in all proportions and highly soluble (>50%) in methanol, isopropanol ethylene glycol. The active ingredient is stable in neutral, alkaline and acidic media.

18.2. Long-chain alkylamines

Long-chain alkylamines and corresponding ammonium salts, e.g. benzoates, lactates, salicylates, hydrohalides, can be characterized as cationic surface active agents, too. They exhibit considerable antimicrobial activity if the alkyl chain consists of 12 to 15 carbon atoms; this is also valid for long-chain aliphatic diamines.

As the long-chain alkylamines meant here in general derive from fatty acids, they are also termed fatty amines, or more correctly fatty C_8 – C_{22} alkylamines. A processing technic is the catalytic dehydration of fatty alcohols at 210–260°C with ammonia or shortchain alkylamines or dialkylamines. Another manufacturing process starts from fats or oils which are transferred to fatty acid amides with ammonia; dehydration of the amides leads to nitriles the catalytic hydrogenation of which produces fatty amines:



Disinfectants containing long-chain alkylamines to be used in the food industry, e.g. in bottling plants, are described by Falter et al. (1991).

Microbicide group (substance class)	18.2. LONG-CHAIN ALKYLAMINES
Chemical name	18.2.1. Dodecylamine
Chemical formula	$C_{12}H_{27}N$
Structural formula	$H_3C-(CH_2)_{11}-NH_2$
Molecular mass	185.36
CAS-No.	124-22-1
EC-No.	204-690-6
EPA TSCA	Section 8 (B) Chemical Inventory
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	Laurylamine, 1-aminododecane
Supplier	FLUKA, THOR (lactate/salicylate mixture)
Chemical and physical properties	
Appearance	colourless mass
Content (%)	min. 99.5 ($H_2O < 0.2$)
Boiling point/range °C (101 kPa)	247–249
Melting point °C	27–29
Density g/ml (20°C)	0.806
Vapour pressure hPa (170°C)	85.12
Flash point °C	115

pH (1% in H ₂ O)	10–12
Ionicity	cationic
Stability	heat resistant, light stable, in acid media formation of dodecylammonium salts; stable up to pH 12
Solubility	low solubility in H ₂ O; soluble in alcohols
Toxicity data	
LD ₅₀ oral	1020 mg/kg rat
	1160 mg/kg mouse
LD ₅₀ intraperitoneal	50 mg/kg mouse
In tests with rabbits 750 µg (24 h) caused severe skin irritation; 50 µg (24 h) produced severe eye irritation.	
Chemical and physical properties of a combination of dodecylammonium salts of lactic acid (8.14.) and salicylic acid (8.10.).	
Appearance	pale yellow, slightly hazy liquid with a mild, characteristic odour
Content %	40–45
Density g/ml (20°C)	0.960–0.980
pH (20°C)	6–6.5
Stability	light stable, stable in the pH range 3 to 10, heat-resistant up to 100°C
Solubility	fully soluble in water and alcohols
Toxicity data (source: THOR)	
LD ₅₀ oral	> 2000 mg/kg rat
Irritant to skin, mucosa and eyes. Not known to cause skin sensitising effects.	

Antimicrobial effectiveness/applications

The mixture of dodecylammonium lactate and dodecylammonium salicylate possesses a wide spectrum of microbicidal efficacy which covers Gram-positive and Gram-negative bacteria, fungi and algae. It may be used for the preparation of microbicidal wash solutions for the antimicrobial treatment for surface coatings, masonry, brick, wood and plaster. The wash solution should contain 0.5–3% of the mixture (concentrate); a 5% solution may be necessary in cases of particularly severe surface infestation. Other applications: Treatment of industrial water circuits, eradication of dry-rot of timber.

Microbicide group (substance class)	18.2. LONG-CHAIN ALKYLAMINES
Chemical name	18.2.2 Bis(3-aminopropyl)dodecylamine
Chemical formula	C ₁₈ H ₄₁ N ₃
Structural formula	H ₃ C-(CH ₂) ₁₁ -N(CH ₂ -CH ₂ -CH ₂ -NH ₂) ₂
Molecular mass	299.55
CAS-No.	2372-82-9
EC-No.	219-145-8
EPA	TSCA Inventory
Synonym/common name	N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine
Supplier	LONZA
Chemical and physical properties	
Appearance	colourless to yellowish liquid with ammoniacal odour
Content (%)	>98 (H ₂ O: max. 1.5)
Stock point °C	approx. 10
Density g/ml (20°C)	0.8650
Viscosity mPa·s (23°C)	38
Surface tension mN/m (20°C)	32 (1% aqueous solution)
pH (1% in H ₂ O)	10–12
Flash point °C	>100

Stability	stable at normal conditions; compatible with nonionic, cationic and selected anionic surfactants; not compatible with aldehydes
Solubility	soluble in water and polar organic solvents
Toxicity data (source: LONZA)	
LD ₅₀ oral	261 m/kg rat
LD ₅₀ dermal	>600 mg/kg rat
In tests with rabbits corrosive to the skin (exposure time: 3 min). Not sensitizing in the guinea pig test.	
Mutagenicity: Ames-test negative.	
Ecotoxicity:	
Biological degradation: 91% within 28 days (OECD-Method 302 B – biologically well degradable).	
LC ₅₀ for Rainbow trout	0.68 mg/l (96 h)
EC ₅₀ for Daphnia magna	0.64 mg/l (24 h)
ErC ₅₀ for algae (Selenastrum sp.)	0.04 mg/l (72 h) – growth inhibition
EC ₅₀ for activated sludge bacteria	18 mg/l (3 h)

Antimicrobial effectiveness/applications

The long-chain alkylamine exhibits microbicidal activity against a broad spectrum of Gram-positive and Gram-negative bacteria, Tb bacteria included. Virucidal activity against enveloped viruses (e.g. Hepatitis B) has been demonstrated too. Algae are highly sensitive to the surface active amine (algaecidal concentration for *Chlorella vulgaris*: 5 mg/l). Hence it is understandable that the long-chain alkylamine is used as an active ingredient in disinfectants and disinfectant cleaners for hospitals, food industry, industrial kitchens, for the antimicrobial treatment of textile material, for the inhibition of the proliferation of algae, aerobic and anaerobic micro-organisms in aqueous systems.

Microbicide group (substance class)	18.2. LONG-CHAIN ALKYLAMINES
Chemical name	18.2.3. Fatty amine hydrochlorides (C ₈ –C ₁₈) derived from coconut oil: 5% Caprylyl, 7% Capryl, 56% Lauryl, 18% Myristyl, 7% Palmityl, 5% Stearyl, 2% Linoleyl
EC	classified and marked in accordance with Directives
EPA-Reg.	for antimicrobial application
Supplier	ISP/CREANOVA
Chemical and physical properties	
Appearance	nearly colourless and odourless fluid
Content (%)	25
Boiling point/range °C (101 kPa)	100
Density g/ml (25°C)	0.96–0.99
Vapour pressure hPa (20°C)	23 (solvent)
Flash point °C	64
Ignition temperature °C	270
Upper flammability limit % v/v i. air	14
Lower flammability limit % v/v i. air	1.1
pH (1% in H ₂ O)	6.8–7.2
Ionicity	cationic
Stability	stable under normal conditions
Solubility	completely soluble in water, soluble in low molecular weight alcohols
Toxicity data (source: DEGUSSA)	

Caustic effect on skin and mucous membranes; corrosive to the eyes with the danger of severe eye injury. Sensitization possible through skin contact.

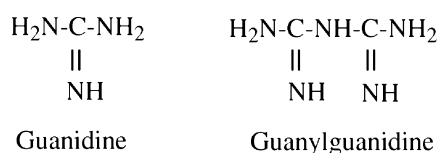
Antimicrobial effectiveness/applications

The stable product is able to protect aqueous raw materials, e.g. resin emulsions, as well as corresponding finished products against microbial deterioration during storage in the wet state (in the unopened container and during the wet products useful service life).

Recommended addition rates: 0.2–0.3%.

18.3. Guanidines and biguanides

Guanidine and guanylguanidine (biguanide) are bases which are stabilized by resonance. They exhibit strong alkaline reaction in aqueous media and form salts with acids. Alkyl derivatives dispose of distinctive antimicrobial efficacy.



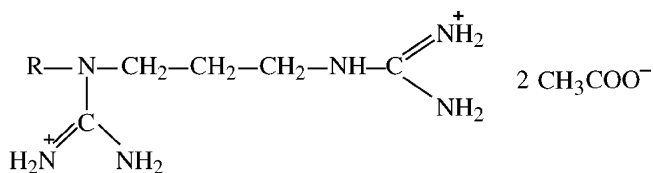
Microbicide group (substance class)

Chemical name

Structural formula

18.3. GUANIDINES AND BIGUANIDES

18.3.1 Cocospropylenediamine-1,5-bis-guanidiniumacetate



R = cocoalkyl: mainly lauryl (C₁₂H₂₅), myristyl (C₁₄H₂₉), palmityl (C₁₆H₃₃)

Average molecular mass

460

CAS-No.

85681-60-3

EC-No.

288-198-7

EPA TSCA

Inventory list

Supplier

LONZA

Chemical and physical properties

Appearance

clear, light yellow, viscous liquid with ammoniacal odour

Content (%)

~78 [isopropanol (1.3.) ~11 ; H₂O max. 13]

Density g/ml (20°C)

1.00

Viscosity mPa·s (50°C)

180–280

Flash point °C

38

Ionicity

cationic

pH (1% in H₂O)

6–7

Stability

compatible with non-ionic surfactants and other QAC's;
incompatible with anionic detergents

Solubility

miscible with water, highly soluble in lower alcohols

Toxicity data (source: LONZA)

LD₅₀ oral

500–2000 mg/kg rat

In tests with rabbits corrosive to skin and eyes.

Ecotoxicity:

LC₅₀ for Zebra fish

0.1–1.0 mg/l (96 h)

Biodegradability: 80% within 28 days (OECD Confirmatory Test).

Antimicrobial effectiveness/application

The N-coco alkyl guanidine derivatives exhibit a broad spectrum of microbicidal activity comprising Gram-positive and Gram-negative bacteria. Remarkable is the high efficacy of the microbicide in presence of organic load. It is used as an active ingredient in general purpose disinfectants.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Vapour pressure hPa (20°C)

Stability

Solubility

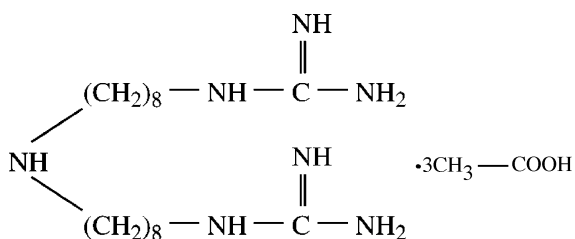
Toxicity data (source: SHELL CHEMIE)

LD₅₀ oral

Carcinogenicity and teratogenicity were not demonstrable in 2 years feeding studies (up to 200 mg/kg diet) with rats.

18.3. GUANIDINES AND BIGUANIDES

18.3.2. Bis(guanidinooctyl)amine triacetate

C₂₄H₅₃N₇O₆

535.74 (free amine: 355.58)

57520-17-9

No EC-no.

1,1-[Imino bis-(octamethylene)]biguanidine triacetate,
Guazatine

SHELL CHEMIE

colourless, crystalline solid; the free base (amine) is a liquid
min. 93

approx. 60

non volatile

decomposition starts at 120°C; stable in neutral and
aqueous acidic media; instable in alkaline media
highly soluble in H₂O and polar organic solvents

Antimicrobial effectiveness/applications

Guazatine as a membrane-active microbicide exhibits a broad spectrum of effectiveness. It is active by interfering with membrane structures of the microbial all. The compound has been developed 1968 by MURPHY CHEMICALS as a fungicide for seed protection. But Guazatine later on also found some usage in wood preservation for the temporary protection of freshly cut and sawn timber.

Microbicide group (substance class)

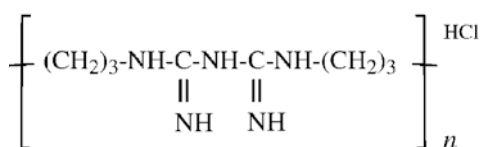
Chemical name

Chemical formula (monomer)

Structural formula

18.3. GUANIDINES AND BIGUANIDES

18.3.3. Poly(hexamethylenebiguanide) hydrochloride (PHMB)

C₈H₁₈ClN₅

average n = 12

Molecular mass (monomer)	219.72
CAS-No.	91403-50-8
EC-Notification-No.	[N 354]; EEC-no. 28
EPA TSCA	Inventory
Synonym/common name	Polyaminopropyl biguanide, Polyhexanide
Supplier	AVECIA, LONZA, THOR
Chemical and physical properties	
Appearance	colourless to yellowish, limpid to slightly opalescent liquid
Content (%)	20 (H ₂ O approx. 80)
Boiling point/range °C (101 kPa)	102–103
Solidification point °C	8–10
Density g/ml (20°C)	1.03–1.05
Viscosity mPa·s (20°C)	1.50
Surface tension mN/m (20°C)	57
(1% in H ₂ O)	
Flash point °C	boils without flashing
Ionicity	cationic
pH	4–5
Stability	exposure to temperatures > 80°C for prolonged periods adversely affects the microbicidal properties; in a concentration of 1 g/litre stable at pH values up to 10.0; above this precipitation may occur. The cationic compound gives water-insoluble precipitates with anionic surfactants, e.g. soaps, alkyl sulphates, alkylaryl sulphonates; the a.i. is precipitated also by strong alkalis and complex phosphates; compatible with non-ionic surfactants and QAC's; gives significantly less foam than QAC's
Solubility	soluble in water and lower alcohols and glycol ethers; insoluble in non-polar organic solvents
Toxicity data (source: AVECIA)	
LD ₅₀ oral	>2000 mg/kg rat
Irritant to skin, mucosa and eyes. May cause sensitization by skin contact.	
Studies in animals have shown that PHMB does not represent a carcinogenic or teratogenic risk to man.	
Ecotoxicity:	
LC50 for Rainbow trout	<1 mg/l (96 h)
LC50 for Bluegill sunfish	0.65–0.9 mg/l (96 h)
EC50 for Daphnia	0.18–0.45 mg/l (48 h), immobilization
IC10 for sewage sludge organisms	40 mg/l, inhibition of respiration rate
As a cationic substance PHMB is regarded to be effectively removed in waste water treatment processes by complex formation and adsorption. PHMB has a low potential for bioaccumulation.	

Antimicrobial effectiveness/applications

The antimicrobial properties of PHMB, which is a membrane-active microbicide, have been described by Davis et al. (1968). Due to these properties and its low toxicity it has found particular use as an active ingredient in disinfecting cleansing agents for the food and beverage industry. However, the surfaces to which PHMB is applied should be widely free from organic matter and anionic detergents. The polymeric biguanide PHMB may also be used in pool sanitizers and for the treatment of raw hides against bacterial degradation in the leather industry. As can be seen from Table 141. PHMB is particularly effective against bacteria and less active against fungi. The relatively high activity of PHMB against *Pseudomonas aeruginosa* is especially interesting. In concentrations of 100–500 mg/litre PHMB exhibits bactericidal effectiveness. Quaternary ammonium compounds with their lack of activity against Pseudomonads and also phenolic microbicides may be used in combination with PHMB or bis-biguanides, e.g. Chlorhexidine (18.3.4) for the formulation of disinfectants with an extraordinary broad activity spectrum (Bansemir et al., 1987). Optimum pH range 5–7.

Table 141 Minimum inhibition concentrations (MIC) of PHMB (20%)—Source: AVECIA

Test organism	MIC (mg/l)
Bacteria	
<i>Bacillus subtilis</i>	5
<i>Enterobacter cloacae</i>	20
<i>Escherichia coli</i> 0157:H7	5
<i>Legionella pneumophila</i>	200
<i>Listeria monocytogenes</i>	45
<i>Proteus vulgaris</i>	200
<i>Pseudomonas aeruginosa</i>	100
<i>Pseudomonas putida</i>	25
<i>Salmonella choleraesuis</i>	55
<i>Salmonella typhimurium</i>	8
<i>Staphylococcus aureus</i>	1
<i>Streptococcus faecalis</i>	25
<i>Streptococcus lactis</i>	25
Fungi	
<i>Aspergillus niger</i>	750
<i>Trycophyton mentagrophytes</i>	25
Yeasts	
<i>Endomycopsis albicans</i>	300
<i>Rhodotorula rubra</i>	25
<i>Saccharomyces cerevisiae</i>	100

In the EC list of preservatives which cosmetic products may contain, PHMB is mentioned with a maximum authorized concentration of 0.3%. – EPA-Reg. as a sanitizer for spas.

Concentrated PHMB solutions have a marked corrosive effect on copper and are not compatible with stainless steel. Diluted PHMB solutions are not more corrosive than water and in contrast to quaternary ammonium compounds are low foaming.

Microbicide group (substance class)

18.3. GUANIDINES AND BIGUANIDES

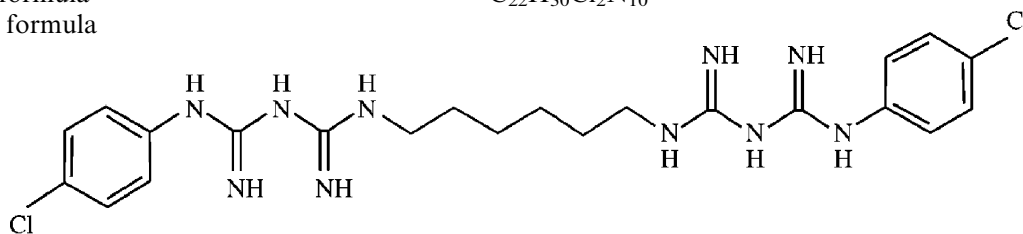
Chemical name

18.3.4. 1,6-Di(4'-chlorophenyldiguanido)hexane

Chemical formula

C₂₂H₃₀Cl₂N₁₀

Structural formula



Molecular mass

505.48

CAS-No.

55-56-1

EC-No.

200-238-7; EEC-no. 42

EPA TSCATS

Data base, Jan. 2001

Synonym/common name

hexamethylenebis[5-(p-chlorophenyl)biguanide],
Chlorhexidine

Supplier

SIGMA-ALDRICH

Chemical and physical properties

Appearance

faintly creme coloured powder

Melting point °C

134–136

Reacting in H₂O

strongly alkaline

Ionicity

cationic

Stability

unstable at high temperatures; incompatible (inactivation) with anionic surfactants; formation of insoluble salts with phosphates, borates, citrates, carbonates, sulphates, chlorides, incompatible with sodium alginate and gums; water soluble salts are formed with digluconic acid (18.3.4a.), acetic acid (18.3.4b.); the dihydrochloride of chlorhexidine (18.3.4c.) disposes only of limited solubility in water

Solubility g/l

0.08 in H₂O

Toxicity data

LD ₅₀ oral	9200 mg/kg rat 2515 mg/kg mouse
LD ₅₀ intraperitoneal	60 mg/kg rat 44 mg/kg mouse
LD ₅₀ subcutaneous	>1000 mg/kg rat 632 mg/kg mouse
LD ₅₀ intravenous	21 mg/kg rat 24 mg/kg mouse

Irritant to skin, mucosa and eyes. Produces photosensitization.

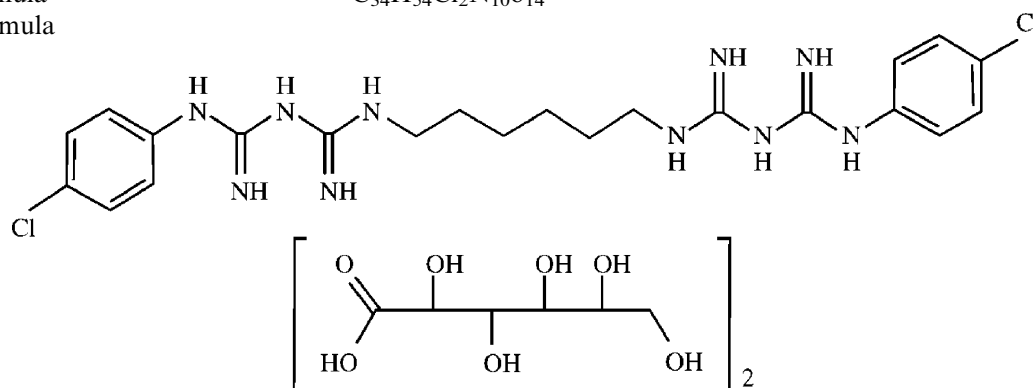
Antimicrobial effectiveness/applications

At low concentrations up to 200 mg/litre the bis-bigunaide Chlorhexidine acts as a bacteriostat by inhibition of membrane enzymes, thus promoting the leakage of cellular constituents. Higher concentrations of Chlorhexidine (>200 mg/litre) coagulate cytoplasmic constituents accompanied by a bactericidal effect (Hugo & Longworth, 1964, 1966). The spectrum of activity covers Gram-positive and Gram-negative bacteria and at higher concentrations fungi, too. Chlorhexidine is not sporicidal and not lethal to acid-fast bacteria or viruses. The optimum pH range for the effectiveness of Chlorhexidine is 5–8, preferably 7. Chlorhexidine is mainly used as an active ingredient in disinfectants, deodorants and antiseptics and as a preservative in cosmetics and pharmaceuticals. The EC positive list of preservatives permitted for use in cosmetics mentions Chlorhexidine and its salts with a maximum authorized concentration of 0.3% expressed as Chlorhexidine. Formulating and using the active ingredient one has to remember its cationic nature which causes reduction of activity in the presence of organic matter, e.g. blood, serum, soaps and other anionic compounds.

Chemical name 18.3.4a. Chlorhexidine digluconate

Chemical formula C₃₄H₅₄Cl₂N₁₀O₁₄

Structural formula



Molecular mass

897.78

CAS-No.

18472-51-0

EC-No.

242-354-0; EEC-no. 42

Synonym/common name

1,1'-hexamethylenebis[5-(4-chlorophenyl)biguanide]digluconate

Supplier

AVECIA

Chemical and physical properties of a 20% aqueous solution

Appearance

almost colourless to pale yellow liquid

Boiling point/range °C (101 kPa)

approx. 105

Density g/ml (20°C)

1.06–1.07

Flash point °C

boils without flashing

pH at 25°C

5.5–7.0

Ionicity

cationic

Stability

stable under normal conditions of storage; limited stability with anionic surfactants, alkyl sulphonates and inorganic salts;

Solubility

miscible with water; soluble in acetone, ethanol

Toxicity data (source: AVECIA) Irritant to skin, eyes and mucous membranes. Chronic effects are unlikely.

Ecotoxicity:

LC₅₀ for Rainbow trout 3.2 mg/l (96 h)

Toxic to sewage organisms. Biochemical oxygen demand (BOD 5): 0.0 g O₂/g. The substance shows no evidence for biodegradability in water.

Antimicrobial effectiveness/applications

The minimum inhibition concentrations listed in Table 142 show that the Chlorhexidine digluconate formulation disposes of a broad efficacy spectrum. It may be utilized as a preservative in cosmetics, as an active ingredient in handwashes and scrubs, for dental and veterinary applications. – Percentage of use in US formulations: chlorhexidine digluconate 0.09%; chlorhexidine acetate 0.01%; chlorhexidine dihydrochloride 0.02%.

Table 142 Minimum inhibition concentrations of a 20% aqueous solution of Chlorhexidine Di-gluconate (Source: AVECIA)

Test organism	MIC (mg/l)
Bacteria	
<i>Enterobacter cloacae</i>	62.5
<i>Escherichia coli</i>	0.5
<i>Klebsiella pneumoniae</i>	15.5
<i>Proteus mirabilis</i>	64
<i>Proteus vulgaris</i>	2
<i>Pseudomonas aeruginosa</i>	31.25
<i>Pseudomonas cepacia</i>	16
<i>Pseudomonas fluorescens</i>	4
<i>Serratia marcescens</i>	16
<i>Staphylococcus aureus</i>	1
<i>Staphylococcus faecalis</i>	32
<i>Staphylococcus mutans</i>	2.5
Fungi/Yeasts	
<i>Aspergillus niger</i>	16
<i>Candida albicans</i>	8
<i>Penicillium notatum</i>	16
<i>Saccharomyces cerevisiae</i>	1

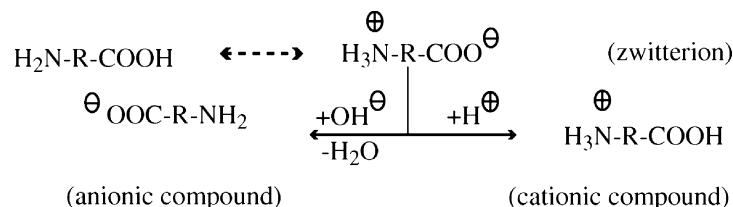
Chemical name	18.3.4b. Chlorhexidine diacetate
Chemical formula	C ₂₆ H ₃₈ Cl ₂ N ₁₀ O ₄
Molecular mass	625.59
CAS-No.	56-95-1
EC-No.	200-302-4; EEC-no. 42
Appearance	colourless crystals
Melting point °C	154–155
Solubility g/l (20°C)	19 in H ₂ O; soluble in ethanol, glycerin, glycols;
Stability	aqueous solutions decompose at temperatures >70°C
Chemical name	18.3.4c. Chlorhexidine dihydrochloride
Chemical formula	C ₂₂ H ₃₂ Cl ₄ N ₁₀
Molecular mass	578.40
CAS-No.	3697-42-5
EC-No.	223-026-6; EEC-no. 42
Appearance	colourless crystals
Melting point	260–262 (decomposition)
Solubility g/l (20°C)	0.6 in H ₂ O; highly soluble in ethanol and glycols
Chemical name	18.3.4d. Other Chlorhexidine salts

Other Chlorhexidine salts which are especially active against Pseudomonads are described by Warner et al. (1980), without, however, having reached significant importance up to today:

Chlorhexidine dinalidixinate	mp 224–26°C
Chlorhexidine diphophanilate dihydrate	mp 172–74°C
Chlorhexidine disorbate monohydrate	mp 100–04°C

18.4. Ampholytes

Ampholytes are amphoteric compounds disposing of acid and basic hydrophilic groups able to accept (acceptor) or to donate (donor) protons. In strong acidic media they behave as bases (cationic), in strong basic media as acids (anionic). Examples are amino acids (betaines) which in water dissociate to zwitterions:



Characteristic for zwitterions is that they at a certain pH value (in general approx. 4) appear externally without charge (point of zero charge or isoelectric point).

Amino acids bearing a long-chain alkyl group at the amino group are amphoteric tensides/detergents which exhibit distinct and broad antimicrobial effectiveness. Their bactericidal activity grasps gram-positive and gram-negative bacteria, including mycobacteria which are not safely killed by other surface active agents such as quaternary ammonium compounds. Amphoteric microbicides are also effective on lipophilic viruses but not on hydrophilic ones. They are applied in disinfecting cleansing agents to be used in hospitals and medical practices and in the food, beverage and cosmetic processing industry. In these applications the active ampholytes profit from having low toxicity, good skin compatibility and from being non-corrosive, odourless and relatively resistant to inactivation by proteins. The antimicrobial activity of the ampholytes remains virtually constant over a wide pH range.

A wide variety of amphoteric microbicides is known; as they are all very similar in properties and activity some important ones only are described here.

Microbicide group (substance class)	18.4. AMPHOLYTES
Chemical name	18.4.1. Dodecyl-di(aminoethyl)glycine
Chemical formula	$\text{C}_{18}\text{H}_{39}\text{N}_3\text{O}_2$
Structural formula	$\text{H}_3\text{C}-(\text{CH}_2)_{11}-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_2-\text{NH}-\text{CH}_2-\text{COOH}$
Molecular mass	329.51
CAS-No.	6843-97-6
EC-No.	229-930-7
Synonym/common name	Lauryldiethylenetriaminoacetic acid, Dodicin
Supplier	CLARIANT
Chemical and physical properties	
Appearance	Clear yellow liquid
Solubility	miscible with water
Toxicity data	
LD ₅₀ oral	3000 mg/kg rat
Non-toxic diet level for rats	300 mg/kg diet (application period 2 years)
ADI value	0.15 mg/kg/day
Not carcinogenic.	
1% aqueous solutions do not cause skin irritation.	

Antimicrobial effectiveness/applications

Dodecyl-di(aminoethyl)glycine is a broad spectrum microbicide. A 1% solution is microbicidal for bacteria, including *Pseudomonads* and *Mycobacterium tuberculosis*, yeasts and fungi within 1–60 min. The microbicidal ampholyte is not compatible with anionic and non-ionic detergents; its antimicrobial effectiveness is, however,

only partly reduced in the presence of proteins. Dodecyl-di(aminoethyl)glycine is an active ingredient in disinfectants preferably for the use in the food industry. It is also used as a preservative for certain pharmaceuticals. The optimum pH range for the antimicrobial activity of the ampholyte is 5–9.

Chemical name	18.4.2. n-Dodecyl- β -aminoprionic acid
Chemical formula	$C_{15}H_{31}NO_2$
Structural formula	$H_3C-(CH_2)_{11}-NH-CH_2-CH_2-COOH$
Molecular mass	257.42
CAS-No.	1462-54-0
Synonym	Dodecyl- β -alanine
Chemical name	18.4.3. N-Dodecyl- β -animobutyric acid
Chemical formula	$C_{16}H_{33}NO_2$
Structural formula	$H_3C-(CH_2)_{11}-NH-\underset{\substack{ \\ CH_3}}{CH}-CH_2-COOH$
Molecular mass	271.45
CAS-No.	10024-28-9

19. Organometallic compounds

Among the microbicidal organometallic compounds, the organomercury and organotin compounds are those which should be mentioned first, for they are the ones that have been used for material protection on a large scale throughout the world. Today, however, there is a strong movement throughout in favour of their substitution in view of their toxicity and especially their ecotoxicity. Organomercury compounds, in particular, have already been largely substituted and are no longer of much importance as microbicides for material protection. In the truest sense of the word they may be termed biocides, since they are effective not only against microbes, but also against all forms of life. Although Hippocrates already knew of the toxicity of mercury compounds he could not anticipate the environmental problems that were to arise through their excessive use – problems rooted in the fact that these compounds are biodegradable only to the stage of methyl- or dimethylmercury, both of which are extremely toxic (Lakowitz & Anderson, 1980). They diffuse rapidly across permeability layers such as membranes. Methyl- and dimethylmercury therefore accumulate in living organisms, especially those at the end of the food chain, such as predatory fish and animals dependent on fish. Without this accumulation the lethal doses of mercury compounds would be less problematical. If a quantity of mercury compounds is absorbed by a human, about 10% of it is carried to the brain. Long-term exposure to mercury compounds is particularly dangerous, even accumulation at very low rates causes manifest damage to the brain and nervous system after a number of years.

Mercury compounds, even at extremely low concentrations, are environmentally unsafe for another reason: investigations have shown that at concentrations as low as 1 ppb mercury-based microbicides cause 50% inhibition of the photosynthesis of phytoplankton and that at 50 ppb this photosynthesis stops entirely (Harries et al., 1970). Concentrations of this order are also toxic to fish.

The arguments against the use of organomercury compounds go beyond toxicity and ecotoxicity; the chemico-physical properties of these products are not particularly favourable either. For example, organomercury compounds are not ideal as paint film fungicides because they are too volatile and insufficiently stable to light. Furthermore, by reacting with H_2S in the atmosphere – in industrial regions, for example, but also in agricultural regions – they make paint films turn grey.

Organotin compounds, though less toxic to warm-blooded animals than organo-mercury compounds, are also very toxic in the environment. Even at concentrations in the ppb range they disturb the biocenosis of surface waters considerably and therefore have to be handled with the greatest care (Polster & Halacka, 1971). They are sensitive to light, but in their main applications, wood preservatives and antifouling paints, this does not matter. Yet their ecotoxicity has led to worldwide demands for a tin ban where antifouling paints are concerned. For the reasons just mentioned, scientists throughout the world began to look for less toxic, yet effective, active ingredients for these paints.

Among the organometallic compounds whose substitution is being demanded for reasons of toxicity is also the arsenic compound 10,10'-oxy(bisphenoxy)arsine (19.1.), a microbicide which has been used predominantly throughout the world to make plastics resistant to microbes.

Because, among organometallic compounds, mercury, tin and arsenic compounds are becoming increasingly less important as microbicides for material protection, only the most important prototypes of these compounds will be described in detail below.

Microbicidal metal chelates are described elsewhere:

13.1.3b.	Zinc-bis(2-thiopyridine-N-oxide)
13.1.3c.	Copper-bis(2-thiopyridine-N-oxide)
13.3.3a.	Copper-8-quinolinolate

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA Reg.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Density g/ml (25°C)

Vapour pressure hPa (25°C)

Stability

Solubility

Toxicity data (source: MORTON THIOKOL)

LD₅₀ oral

dermal

Corrosive to skin, mucosa and eyes.

Rats exposed for 2 weeks to vapours of OBPA heated to 121°C showed no signs of irritation of nose and eyes.

Ecotoxicity:

Toxicity of a 10% solution of OBPA in propylene glycol for trout: LC₅₀ 0.2 mg/l (96 h).

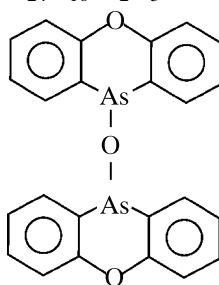
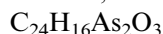
Chemically OBPA can be regarded as virtually insoluble in water. However, leaching, diffusion and solution in water is not excluded, so that OBPA may be detected in the environment.

Antimicrobial effectiveness/applications

OBPA is a biocide, that means it is not only toxic to microbes but also to fish and other living species. Its antimicrobial effectiveness is similar to that of organo-mercurials covering a broad spectrum of bacteria, yeasts and mould producing fungi (minimum inhibition concentrations approx. 10 mg OBPA/litre). Among the fungal species *Scopulariopsis brevicaule* is relatively resistant to OBPA under use conditions.

19. ORGANO METALLIC COMPOUNDS

19.1. 10, 10'-Oxybisphenoxyarsine (OBPA)



502.24

58-36-6

200-377-3

approval for antimicrobial application

disphenoxyarsin-10-yl oxide

MORTON THIOKOL INC.

white, odourless, crystalline powder

~100 (As content: 29.84)

184–186

1.40–1.42

10⁻³

heat resistant under processing conditions for plastic material, e.g. PVC; thermal decomposition range 300–380°C

in H₂O: 5 mg/l; soluble in organic solvents, e.g. propylene glycol, butylbenzyl phthalate, di(2-ethylhexyl) phthalate, diisodecyl phthalate, epoxidized soya-oil

30–40 mg/kg rat

100–200 mg/kg rat

OBPA is mainly used for the antimicrobial treatment of plastic material. For that purpose it is offered as a solution in plasticizers, e.g. 2% solutions in epoxidized soja-oil or diisodecyl phthalate or di(2-ethylhexyl) phthalate or polypropylene glycol or butylbenzyl phthalate. Such formulations are easily incorporated into master batches for the production of plastic materials without negatively influencing the properties of the finished products. OBPA is sufficiently heat resistant to stand the processing temperatures. It is recommended to incorporate 1.5% of one of the a.m. 2% OBPA solutions into plastic material for interior application, that is 0.03% OBPA or 0.009% As only. With regard to the small amounts of OBPA which achieve antimicrobial effectiveness in plastic materials, leachability of active ingredient may be a problem, although the solubility of OBPA in water is very low. For exterior application the recommendation therefore is addition of 2.5% of a 2% OBPA solution. Upsher & Roseblade (1984) report about the limited long-term activity of OBPA in plastic material exposed at a jungle site.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility (g/l)

Toxicity data

LD₅₀ oral

LD₅₀ subcutaneous

LD₅₀ intraperitoneal

LD₅₀ intravenous

100 µg (24 h) cause severe irritation on the human skin. 50 µg (24 h) create severe eye irritation in tests with rabbits. PMA may be resorbed by the skin and may cause sensitization.

Exposure limits (occupational) (mg Hg/m³)

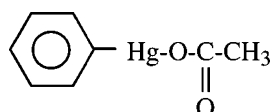
Ecotoxicity:

PMA is highly ecotoxic. Accumulation in sea/water animals.

19. ORGANOMETALLIC COMPOUNDS

19.2 Phenylmercury acetate (PMA)

C₈H₈HgO₂



336.74

62-38-4

200-532-5; EEC-No. 17.

Data base, Jan. 2001

(acetoxymcury)benzene

COSAN, SIGMA-ALDRICH

crystalline, colourless powder

~100 (Hg content: 59.6)

150–152

Sensitive to strong oxidation and reducing agents, and acids; due to its electrophilic character PMA reacts with anionic detergents and sulphides under inactivation
1.7 in H₂O; soluble in ethanol, acetone, benzene

41 mg/kg rat

13 mg/kg mouse

12 mg/kg mouse

13 mg/kg mouse

18 mg/kg mouse

Germany, France 0.01
NL, UK 0.05

Antimicrobial effectiveness/applications

PMA is extremely effective against bacteria, fungi, yeasts and algae, it acts also sporistactically. The minimum inhibition concentrations for bacteria range between 5 and 15 mg/litre and those for fungi, yeast and algae below 1 mg/litre. In acidic media PMA is not particularly effective; the optimum pH for activity of PMA is approximately 8.8, as at higher pH values dissociation of PMA to the electrophilic active radical Ph-Hg⁺ increases. The radical is likely to interfere with a large number of cell processes by binding, for example, to essential thiol groups thus inhibiting glycolysis. But PMA and other organomercury compounds also show a strong affinity for phosphates, histidine side chains of proteins and for purines, pteridines and porphyrines (Corbett et al., 1984). It

is therefore not surprising that organomercury compounds, unlike most other microbicides, also inhibit exogenic enzymes, e.g. cellulases.

PMA has been used widely as a microbicide for the in-can/in-tank protection of aqueous functional fluids such as latex paints. The ability of PMA to inhibit enzymes that break down cellulosic thickeners was considered a special advantage in that application. At higher addition rates PMA acts also as a paint film fungicide and algicide. However, the duration of activity of PMA in paint films is limited due to volatility and leachability of the active ingredient. Additionally one risks discolouration. PMA was also used extensively in slimicides, algicides, antifouling paints and wood preservatives, where it also exhibited insecticidal effectiveness.

In the EEC list of preservatives which cosmetic products may contain the use of PMA is limited to eye make-up and eye make-up remover only; max. concentration: 0.007% (of Hg). Such products must be labeled: "Contains Phenylmercury Compounds". – Percentage of use in US cosmetic formulations, 0.17% PMA, if no other effective and safe (Hg-free) preservative is available.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

SPA TSCATS

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility

Toxicity data

LD₅₀ oral

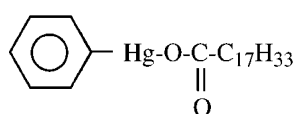
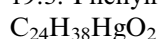
Irritant to skin, mucosa and eyes.

Occupational exposure limits (mg Hg/m³)

PMO is highly ecotoxic.

19. ORGANOMETALLIC COMPOUNDS

19.3. Phenylmercury oleate (PMO)



559.16

104-60-9

203-218-6

Data base, Jan. 2001

Phenylmercury (II) oleate

COSAN

white to yellow powder

~100 (Hg content: 35.9)

186–215

stable between pH 4 and 9; colouration and inactivation with sulphides

Virtually insoluble in H₂O; dissolves readily in mineral spirits and aromatic solvents

approx. 60 mg/kg rat

Germany, France 0.01

NL, UK 0.05

Antimicrobial effectiveness/applications

PMO is in efficacy similar to PMA. As PMO is virtually insoluble in water and of low volatility only, it has been used mainly as an active ingredient in wood preservatives, antifouling coatings, exterior and interior latex paints and sealants. As small amounts of mercury compounds are toxic to many plants, it is recommended not to use PMO in greenhouse paints.

Microbicide group (substance class)

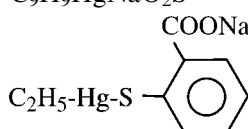
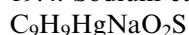
Chemical name

Chemical formula

Structural formula

19. ORGANOMETALLIC COMPOUNDS

19.4. Sodium ethylmercury thiosalicylate



Molecular mass	404.82
CAS-No.	54-64-8
EC-No.	200-210-4; EEC-No. 16
EPA TSCA	Section 8(B) Chemical Inventory
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	2-(ethylmercurythiobenzoic) acid sodium salt, Thiomersal
Supplier	FLUKA, ELI LILLY
Chemical and physical properties	
Appearance	creme-coloured crystalline powder
Content (%)	≥95 (Hg content: 49.6)
Melting point °C	234–237
pH value	7–8 (1% solution in H ₂ O: 6.7)
Flash point °C	>250
Stability	sensitive to light and oxygen, incompatible (inactivation) with non-ionic and cationic compounds, lecithin, thioglycollate, and proteins; unstable in the presence of potassium iodide
Solubility g/l	1000 in H ₂ O; 100 in methanol; 120 in ethanol; virtually insoluble in ether, benzene
Toxicity data	
LD ₅₀ oral	75 mg/kg rat 91 mg/kg mouse
LD ₅₀ subcutaneous	98 mg/kg rat 66 mg/kg mouse
LD ₅₀ intraperitoneal	54 mg/kg mouse
LD ₅₀ intravenous	45 mg/kg mouse
Tumorigenic (neoplastic by RTECS criteria). 8 µg cause mild irritation on the rabbit eye.	
Occupational exposure limits (mg Hg/m ³)	France, Germany 0.01 Japan, UK 0.05
Ecotoxicity:	
Highly hazardous to the environment. Toxic to aquatic animals.	

Antimicrobial effectiveness/applications

The reaction of thiosalicylic acid with ethylmercury chloride in aqueous NaOH leads to Thiomersal which is highly effective against Gram-positive and Gram-negative bacteria, moulds and yeast. It is mainly used as a preservative in pharmaceutical preparations; addition rates: 100–200 mg/litre. Optimum pH range: 6–8. In the EC list of preservatives allowed for cosmetics Thiomersal is mentioned with a maximum authorized concentration of 0.007% (of Hg) for eye make-up and eye make-up remover only; on the label there must be printed the warning: Contains Thiomersal. Percentage of use in US cosmetic formulations 0.1%.

Microbicide group (substance class)	19. ORGANOMETALLIC COMPOUNDS
Chemical name	19.5. Bis(tributyltin) oxide (TBTO)
Chemical formula	C ₂₄ H ₅₄ OSn ₂
Structural formula	$ \begin{array}{c} \text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2 \\ \diagdown \quad \quad \diagup \\ \text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2 \quad \text{---} \text{Sn-O-Sn} \quad \text{---} \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3 \\ \diagup \quad \quad \diagdown \\ \text{H}_3\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_2 \quad \text{---} \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3 \end{array} $
Molecular mass	596.07
CAS-No.	56-35-9

EC-No.	200-268-0
EPA Reg.	for antimicrobial application
EPA TSCA	Section 8(B) Chemical Inventory
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	Hexabutyldistannoxane
Supplier	INT. SPEC. PRODUCTS, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	clear, straw coloured liquid with a characteristic odour
Content (%)	~95 (Sn content 38.6)
Boiling point/range °C (1.3 kPa)	200–230 (180 at 0.27 kPa)
Solidification point °C	< -45
Density g/ml (20°C)	1.17
Vapour pressure hPa (20°C)	approx. 1×10^{-5}
Surface tension mN/m (25°C)	30.6
Refractive index nD (20°C)	1.4865–1.4870
Flash point °C	168–179
Log POW	3.2–3.8
Stability	hydrolysis/hydration to $\text{TBTOH}_2^+ \cdot \text{OH}^-$ in water; the C-Sn bond of TBTO is, however, stable between pH 2.3 and 10.3, but sensitive to UV light (degradation of Bu_3SnOH via $\text{Bu}_2\text{-Sn}^{2+}$ and BuSn^{3+} to Sn^{4+})
Solubility (mg/l)	20–60 in H_2O dist.; 3–10 in artificial seawater; sparingly soluble in ethylene glycol; soluble in most organic solvents
Toxicity data	
LD ₅₀ oral	87 mg/kg rat 55 mg/kg mouse
LD ₅₀ dermal	>300 mg/kg rat 163 mg/kg mouse 900 mg/kg rabbit
LD ₅₀ intraperitoneal	5 mg/kg rat 12.5 mg/kg mouse
LD ₅₀ intravenous	6 mg/kg mouse
LC ₅₀ inhalative (aerosol)	0.064 ml/m ³ (4 h)
Corrosive to skin, mucosa and eyes, but not a skin sensitizer.	
From many test results available up to now there is no evidence of mutagenicity, teratogenicity or carcinogenicity.	
Occupational exposure limits (mg Sn/m ³) France, Germany, UK, USA 0.1	
Ecotoxicity:	
LC ₅₀ for fish	6–24 mg/l (96 h)
LC ₅₀ for oysters	1.6–1.9 mg/l (48 h); growth inhibition starts at 0.02–0.06 mg/l
EC ₅₀ for <i>Daphnia magna</i>	1.7 mg/l (48 h)
NOEC	0.16 mg/l (21 days)
EC ₅₀ for micro-algae	0.33–1.03 mg/l (72 h) in fresh water and sea water
Biodegradation of TBTO by micro-organisms occurs under debutylation to inorganic tin (Sn^{4+}). However, at a concentration of 5 mg TBTO/ml the activity of activated sludge organisms is inhibited already.	

Antimicrobial effectiveness/applications

Although organotin compounds have been known since the middle of the 19th century, their suitability as microbicides for the protection of materials against biodeterioration was not recognized before the middle of the 20th century. Especially TBTO then quickly gained considerable importance for the microbicidal treatment of a variety of materials due to its high activity and broad spectrum of effectiveness which covers bacteria, yeasts, fungi and algae. Tributyltin compounds such as TBTO belong to the electrophilic active microbicides and work for instance by inhibiting oxidative phosphorylation.

The use of TBTO as an active ingredient in technical preservatives and in disinfectants is no longer of significant importance. Approximately 79% of the TBTO and similar derivatives are used in antifouling coatings and

20% in wood preservatives. However, in these important fields of application the use of TBTO is decreasing because of unfavourable toxicity data. In the most significant field of application for organotin – antifouling coatings (Anderson & Dalley, 1986) – the ecotoxicity of the compounds causes severe problems. TBTO was much in demand as an active ingredient for antifouling paints also because of its activity against different sea animals which attach to ship bottoms.

In the meantime alternatives to TBTO have been developed which are getting more and more importance; worth mentioning are: N-haloalkylthio compounds e.g. 16.5. and 16.6., the 4-isothiazolin-3-one derivative 15.5., inhibitors of photo synthesis e.g. Diuron (10.9.) and triazine derivatives (e.g. 20.6.), tetrachloroisophthalodinitril (17.9.), dithiocarbamates e.g. Zineb (11.12.2.).

In wood preservatives an advantage is the strong efficacy of TBTO against wood destroying fungi (Table 143). As the activity of TBTO against staining fungi, e.g. *Aureobasidium pullulans* and *Sclerophoma pityophila*, is not very pronounced, in wood preservatives TBTO is generally combined with other fungicides, e.g. trihalomethylthio compound (16.), benzimidazolyl methylcarbamate (11.4.), and iodopropargylcarbamate (11.1.), which are highly effective against wood staining fungi. The permanence of TBTO in timber is not unlimited. There may occur losses of TBTO from impregnated timber by evaporation and by degradation in situ, particularly from zones near the surface and from unpainted wood. In the inner parts of impregnated wood TBTO is normally protected against the degrading influence of UV light, but not in any case against fungal detoxification. According to Henshaw et al. (1978) especially the white-rot fungus *Coriolus versicolor* is able to degrade TBTO with the help of an efficient oxidase system.

Table 143 Toxic limits of TBTO, tested in pine-wood according to british standard 838^a

Test fungi	Toxic limits (kg/m ³ /wood)
<i>Coniophora cerebella</i>	0.36–0.81
<i>Lenzites trabea</i>	0.02–0.06
<i>Poria monticola</i>	0.02–0.06
<i>Merulius lachrymans</i>	0.02–0.06
<i>Polystictus versicolor</i>	1.18–1.33

^aSchering AG 1984, Technical Information – Organotin Compounds.

Microbicide group (substance class)
Chemical name

19. ORGANOMETALLIC COMPOUNDS
19.6. Tributyltin esters (TBT esters)

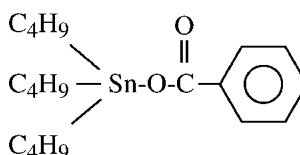
The esterification of bis(tri-n-butyltin) oxide (TBTO) with carboxylic acids leads to tributyltin esters:



Among these, carboxylic acid esters have gained importance in the field of wood preservation, because they are of lower volatility and water-solubility, but higher thermal stability than TBTO. From comparison data it can be concluded that the tributyltin (TBT) moiety of the TBT esters determines their antimicrobial effectiveness and also their oral toxicity. This is in line with the fact that the TBT esters tend to dissociated into the hydrated TBT cation and the respective anion. Due to the lower TBT content compared with TBTO greater quantities of TBT carboxylic acid ester therefore have to be used to achieve sufficient antimicrobial effectiveness. Hydrohalide acid esters of practical importance as microbicides have been tributyltin fluoride (19.6.6.) and triphenyltin chloride (19.6.7.).

Chemical name
Chemical formula
Structural formula

19.6.1. Tributyltin benzoate (TBTB)
 $\text{C}_{19}\text{H}_{32}\text{O}_2\text{Sn}$



Molecular mass

411.18

CAS-No.	4342-36-3
EC-No.	220-120-9
EPA Reg.	approval for antimicrobial application
Synonym/common name	tributylstannyl benzoate
Supplier	INT. SPEC. PRODUCTS

Chemical and physical properties

Appearance	colourless liquid at temperatures above 25°C
Content (%)	approx. 97 (Sn content: 28)
Boiling point/range °C (1.3 kPa)	approx. 135
Density g/ml (20°C)	1.17–1.20
Viscosity mPa·s (25°C)	approx. 20
Solubility	sparingly soluble in H ₂ O; soluble in organic solvents

Toxicity data (source: Schweinfurth & Günzel, 1987)

LD ₅₀ oral	100–200 mg/kg rat 108 mg/kg mouse
LD ₅₀ dermal	508 mg/kg rat
Irritant to skin, mucosa and eyes.	
Occupational exposure limits (mg Sn/m ³)	France, Germany, UK, USA 0.1

Chemical and physical properties of a 45.6% solvent based formulation

Appearance	nearly colourless and odourless liquid
Density g/ml (20°C)	1.080–1.120
Viscosity mPa·s (25°C)	50 maximum
Flash point °C	174
Stability	oil and oleoresinous paint formulations may inactivate TBTB
Solubility	not miscible or difficult to mix with water; soluble in organic solvents

Toxicity data (source: DEGUSSA)

LD ₅₀ oral	174 mg/kg rat
LD ₅₀ dermal	>2000 mg/kg rabbit

Irritating effect on the eyes. – Sensitization possible through skin contact.

Ecotoxicity:

Wastes are acutely hazardous. The biocide is toxic to fish, birds and other wildlife.

Table 144 Minimum inhibition concentration (MIC in mg/l) of a 45.6% TBTB formulation (source: ISP).

Bacterial species		MIC
Gram positive		
<i>Bacillus subtilis</i>	ATCC* 27328	2.5
<i>Bacillus licheniformis</i>	ATCC 27326	5.0
<i>Bacillus megaterium</i>	ATCC 27327	10
<i>Staphylococcus aureus</i>	ATCC 6588	2.5
Gram negative		
<i>Pseudomonas aeruginosa</i>	ATCC 10145	100
<i>Pseudomonas putida</i>	ATCC 21399	100
<i>Escherichia coli</i>	ATCC 8739	>4000
<i>Enterobacter cloacae</i>	ATCC 13047	>4000
Fungal species		
<i>Candida albicans</i>	ATCC 2091	5
<i>Aspergillus niger</i>	ATCC 6275	5
<i>Aspergillus oryzae</i>	ATCC 10191	5
<i>Aureobasidium pullulans</i>	ATCC 9348	5
<i>Gliocladium viren</i>	ATCC 9645	10
<i>Penicillium funiculosum</i>	ATCC 11797	10
<i>Penicillium citrinum</i>	ATCC 9849	5

Antimicrobial effectiveness/applications

There are gaps in the spectrum of effectiveness of TBTB as is demonstrated by the MIC's in Table 144. However, the active agent disposes of a high degree of antifungal activity which enables it to exhibit excellent mildew resistance in a great variety of materials e.g. in coatings and adhesives. Mould resistance of dried films may be achieved by addition rates of 0.2–0.5% of the TBTB formulation, based on total weight of product. If TBTB is used for the in-can protection of water based products it is recommendable to use it in conjunction with other in-can preservatives disposing of high antibacterial activity. If TBTB is used as a paint film fungicide one has to keep in mind its sensitivity to the effect of UV-light.

Microbicide group (substance class)

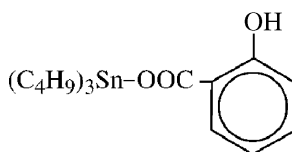
Chemical name

Chemical formula

Structural formula

19. ORGANOMETALLIC COMPOUNDS

19.6.2. Tributyltin salicylate (TBTS)

 $C_{19}H_{32}O_3Sn$ 

Molecular mass

427.18

CAS-No.

4342-30-7

EC-No.

224-397-7

EPA Reg.

approval for antimicrobial application

Synonym/common name

tributylstannyl salicylate

Supplier

INT. SPEC. PRODUCTS

Chemical and physical properties of a 48% solvent based formulation

Appearance

nearly colourless, odourless liquid

Content (%)

48 (tin content: 13.3)

Boiling point/range °C (101 kPa)

274

Density g/ml (20°C)

1.1

Viscosity mPa·s

47 maximum

Flash point °C

204

Ignition temperature °C

251

pH at 20°C

4.7

Stability

some oil and oleoresinous paint formulations may inactivate the organotin compound

Solubility

soluble in most organic solvents, dispersible in water

Toxicity data (source: DEGUSSA)

LD₅₀ oral

134 mg/kg rat

Irritant to skin, mucous membranes and eyes. No sensitizing effects known.

Ecotoxicity:

Extremely hazardous for water; toxic to fish, birds and other wildlife.

Antimicrobial effectiveness/applications

According to its spectrum of efficacy TBTS may be used as a fungicide in the coatings industry. Mould resistance of dried films is achieved by levels of 0.2 to 0.5% TBTS formulations based on total wet weight of product. For protection against in-can fungal attack suggested use levels range from 0.04 to 0.16% TBTS formulation based on finished wet product weight. It is recommended to use an in-can preservative which disposes of antibacterial activity, additionally.

Microbicide group (substance class)

19. ORGANOMETALLIC COMPOUNDS

Chemical name

19.6.3. Tributyltin linoleate (TBTL)

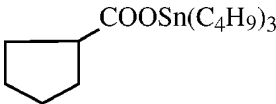
Chemical formula

 $C_{30}H_{58}O_2Sn$

Structural formula	$\text{H}_3\text{C}-(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOSn}(\text{C}_4\text{H}_9)_3$
Molecular mass	569.50
CAS-No.	24124-25-2
EC-No.	246-024-7
Synonym/common name	(Z,Z)-tributyl(octadeca-9,12-dienoyl)stannane
Chemical and physical properties	
Appearance	clear, yellow liquid with a characteristic odour
Content (%)	~98 (Sn content: 20.5)
Boiling point/range °C (1.5 kPa)	~140
Density g/ml (20°C)	1.05–1.06
Viscosity mPa·s (25°C)	~350
Flash point	~105
Volatility	weight loss after storage at 65°C for 6 days: 1% (TBTO for comparison: 26%)
Solubility g/l	0.001 in H ₂ O; soluble in organic solvents
Toxicity data (source: Schweinfurth & Günzel, 1987)	
LD ₅₀ oral	190 mg/kg rat
Irritant to skin, mucosa and eyes.	
Occupations exposure limit (mg Sn/m ³)	France, Germany, UK 0.1

Antimicrobial effectiveness/applications

TBTL is a microbicide of minor importance in the field of material protection; its antimicrobial activity corresponds to that of tributyltin naphthenate (19.6.4.).

Microbicide group (substance class)	19. ORGANOMETALLIC COMPOUNDS
Chemical name	19.6.4. Tributyltin naphthenate/TBTN
Chemical formula	$\text{C}_{18}\text{H}_{36}\text{O}_2\text{Sn}$
Structural formula	
Molecular mass	403.2
CAS-No.	85409-17-2
EC-No.	287-083-9
Synonym/common name	mono(naphthenoyloxy)tributyl stannane
Chemical and physical properties	
Appearance	clear, yellow liquid with a characteristic odour
Content (%) (technical grade)	~71 (Sn content: approx. 21)
Boiling point/range °C (0.05 kPa)	125
Density g/ml (20°C)	approx. 1.09
Viscosity mPa·s (25°C)	approx. 2900
Flash point °C	78
Volatility	weight loss after storage at 65°C for 6 days: 2.9% (TBTO for comparison: 26%)
Solubility g/l	0.0013 in H ₂ O; soluble in organic solvents
Toxicity data (source: Schweinfurth & Günzel, 1987)	
LD ₅₀ oral	225 mg/kg rat
dermal	4600 mg/kg rat
LC ₅₀ for rats on inhalation of aerosol	152 mg/m ³
Occupational exposure limit (mg Sn/m ³)	France, Germany, UK 0.1

Antimicrobial effectiveness/applications

TBTN exhibits fungicidal and algicidal activity and has been used as an active ingredient in antifouling paints and wood preservatives. The fungicidal activity of TBTN directs preferably at wood-destroying fungi. Due to the lower tributyltin content compared with TBTO (19.5.) approximately double the addition rates have to be applied.

Microbicide group (substance class)

Chemical name

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

19. ORGANOMETALLIC COMPOUNDS

19.6.5. Other tributyltin carboxylic acid esters

19.6.5a. Tributyltin oleate

 $C_{30}H_{60}O_2Sn$ $H_3C-(CH_2)_7-CH=CH-(CH_2)_7-COO-Sn(C_4H_9)_3$

571.52

3090-35-5

221-433-3

Chemical name

19.6.5b. Tributyltin acrylate

Chemical formula

 $C_{15}H_{30}O_2Sn$

Structural formula

 $H_2C=CH-COO-Sn(C_4H_9)_3$

Molecular mass

361.12

CAS-No.

13331-52-7

EC-No.

236-381-7

Chemical name

19.6.5c. Tributyltin maleate

Chemical formula

 $C_{28}H_{56}O_4Sn_2$

Structural formula

 $(C_4H_9)_3Sn-OOC-CH=CH-COO-Sn(C_4H_9)_3$

Molecular mass

694.18

CAS-No.

24291-45-0

EC-No.

246-125-6

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

19. ORGANOMETALLIC COMPOUNDS

19.6.6. Tributyltin fluoride (TBTF)

 $C_{12}H_{27}FSn$ C_4H_9

$$\begin{array}{c} \diagdown \\ C_4H_9 - Sn - F \\ \diagup \\ C_4H_9 \end{array}$$
 C_4H_9

Molecular mass

309.06

CAS-No.

27615-98-1

EC-No.

217-847-9

EPA TSCATS

Data base, Jan. 2001

Synonym/common name

fluorotributyl stannane

Chemical and physical properties

Appearance

white powder

Content (%)

>97 (Sn content: ~37)

Melting point °C

269–271 (decomposition)

Bulk density g/l

600

Flash point °C

145

Solubility g/l

48 in methanol, 34 in isopropanol, 100 in chloroform, 9 in xylene; virtually insoluble in H₂O

Toxicity data (source: Schweinfurth & Günzel, 1987)

LD₅₀ oral

94 mg/kg rat

LD ₅₀ dermal	680 mg/kg rabbit
Irritant to skin, mucosa and eyes.	
Occupational exposure limit (mg Sn/m ³)	France, Germany, UK USA 0.1

Antimicrobial effectiveness/applications

TBTF's spectrum of efficacy comprises algae, bacteria, fungi, yeast. It has been used mainly as an active ingredient in antifouling coatings and wood preservatives.

Microbicide group (substance class)	19. ORGANOMETALLIC COMPOUNDS
Chemical name	19.6.7. Triphenyltin chloride (TPTC)
Chemical formula	C ₁₈ H ₁₅ ClSn
Structural formula	(C ₆ H ₅) ₃ Sn-Cl
Molecular mass	385.48
CAS-No.	639-58-7
EC-No.	211-358-4
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	chlorotriphenylstannane, chlorotriphenyltin
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	solid with a characteristic odour
Content (%)	≥97 (Sn content ~30)
Boiling point/range °C (101 kPa)	240
Melting point °C	103–106
Solubility	virtually insoluble in H ₂ O; soluble in most organic solvents
Toxicity data	
LD ₅₀ oral	135 mg/kg rat
LD ₅₀ intraperitoneal	21 mg/kg mouse
LD ₅₀ intravenous	18 mg/kg mouse
In tests with rabbits the application of 100 mg (24 h) caused severe skin and eye irritation. Hazardous to the environment. Toxic to aquatic animals and plants.	
Occupational exposure limits (mg Sn/m ³):	
ACGIH TLV-TWA (USA)	0.1
France, Germany, UK	0.1

Antimicrobial effectiveness/applications

TPTC exhibits strong algacidal activity (see Table 145). It has been used mainly as an active ingredient in marine or freshwater antifouling paints.

Table 145 Minimum inhibition/killing concentration of TPTC against seawater algae in mg/l

Test algae	MIC/MKC
<i>Cladophora</i>	0.10
<i>Enteromorpha</i>	0.05
<i>Intestinalis</i>	1–2

Source: Degussa

Microbicide group (substance class)

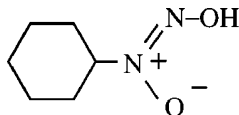
Chemical name

20. VARIOUS COMPOUNDS

20.1. N-Cyclohexyl-N'-hydroxy-diazeniumoxide derivatives-HDO salts

The HDO salts are powerful fungicides. They derive from the basic substance N-cyclohexyl-N'-hydroxy-diazeniumoxide:

Structural formula



Chemical formula

 $C_6H_{12}N_2O_2$

Molecular mass

144.18

CAS-No.

72553-39-0

In contrast to HDO salts the basic substance has not gained importance as a microbicide.

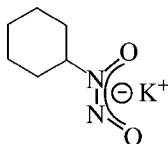
Chemical name

20.1a. (N-cyclohexyldiazeniumdioxy)-potassium (K-HDO)

Chemical formula

 $C_6H_{11}KN_2O_2$

Structural formula



Molecular mass

182.09

CAS-No.

66603-10-9

EC-No.

248-617-6

Supplier

BASF

Chemical and physical properties

Appearance

colourless solid with a typical smell

Density g/ml (20°C)

 1.431 ± 0.01 (relative density)

Solubility g/l (20°C)

452 in H_2O , approx. 540 in ethylene glycol

Toxicity data (source: BASF) of K-HDO techn.; 30% a. i.

LD₅₀ oral

440 mg/kg rat

LD₅₀ dermal

>5000 mg/kg rat

Irritant to skin and mucous membranes. There is no evidence that K-HDO produces cancerogenic or teratogenic effects. – Not mutagenic in genotoxicity tests.

Antimicrobial effectiveness/applications

(N-Cyclohexyldiazeniumdioxy)-potassium is a powerful fungicide which in particular is active against wood destroying fungi. It is mainly used for the protection of wooden materials like plywood and particle board and for those incorporated together with glues and adhesives into such materials. The proportioning rate depends on the intended application for the wood-based panels.

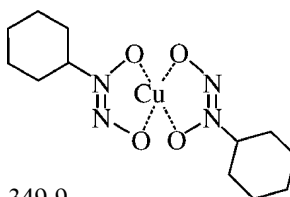
Chemical name

20.1b. Bis-(N-cyclohexyldiazeniumdioxy)-copper (Cu-HDO)

Chemical formula

 $C_{12}H_{22}CuN_4O_4$

Structural formula



Molecular mass

349.9

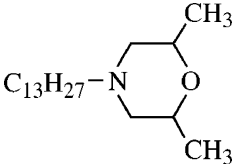
CAS-No.

312600-89-8

EC-Notification-no.	N 005 (A = checked and found acceptable)
Supplier	BASF
Chemical and physical properties	
Appearance	solid blue powder
Melting point °C	approx. 157
Density g/ml (20°C)	1.834 ± 0.01 (relative density)
Vapour pressure hPa (20°C)	$< 1 \times 10^{-6}$
pH (6 mg/l) at 20°C	7
Log POW (25°C)	2.46
Solubility g/l (20°C)	0.0061 in H ₂ O; 2.1 in ethylene glycol, 76 in benzene, 241 in chloroform, 20.5 in methanol
Toxicity data (source: BASF)	
LD ₅₀ oral	380 mg/kg rat
LD ₅₀ dermal	>2500 mg/kg rat
In tests with rabbits not irritant to skin and eyes. Not a skin sensitizer according to the guinea pig test. In various tests Cu-HDO has not shown mutagenic or teratogenic or cancerogenic effects.	

Antimicrobial effectiveness/applications

Cu-HDO disposes of high fungicidal activity. It is recommended for use in liquid wood preservatives providing preventive efficacy against wood destroying fungi including those causing soft rot. Cu-HDO based preservatives are suitable for application by industrial treatment methods, e.g. vacuum pressure and oscillating method in particular for structural timber in interior and exterior use. Examples: Timber in horticultures and landscape gardening, posts, fence, palisades, playground equipment and wood paving.

Microbicide group (substance class)	20. VARIOUS COMPOUNDS
Chemical name	20.2. 2,6-Dimethyl-4-tridecylmorpholine
Chemical formula	C ₁₉ H ₃₉ NO
Structural formula	
Molecular mass	297.53
CAS-No.	24602-86-6
EC-No.	246-347-3
Synonym/common name	Tridemorph
Supplier	BASF, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	yellow liquid with a slight typical odour
Content (%)	approx. 100
Boiling point/range °C (0.07 kPa)	134
Solidification point °C	< -20
Density g/ml (20°C)	approx. 0.87
Vapour pressure hPa (20°C)	1×10^{-4}
Flash point °C	138
Solubility g/l (20°C)	0.1 in H ₂ O; soluble in most organic solvents
Toxicity data	
LD ₅₀ oral	650 mg/kg rat 1560 mg/kg mouse 750 mg/kg rabbit 540 mg/kg cat

Irritant to skin and mucous membranes.

Ecotoxicity: Harzardous to the environment.

Antimicrobial effectiveness/applications

Tridemorph is a broad spectrum microbicide which was developed by BASF 1969 as a systematizing fungicide for plant protection. It may also be used in wood preservatives. At application rates of ≤ 100 mg/l it has been found to be active against wood colouring and wood destroying fungi.

Microbicide group (substance class)

Chemical name

Chemical formula

Structural formula

Molecular mass

CAS-No.

EC-No.

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Boiling point/range °C (7×10^{-3} kPa)

Solubility g/l

Toxicity data

LD₅₀ oral

LD₅₀ dermal

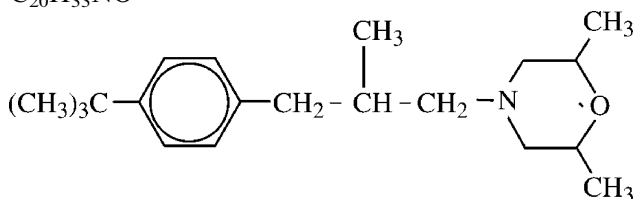
LD₅₀ on inhalation

Irritant to skin and mucous membranes.

20. VARIOUS COMPOUNDS

20.3. (±)-cis-4-[3-(4-tert. Butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine

C₂₀H₃₃NO



303.49

67564-91-4

266-719-9

Fenpropimorph

BASF, SIGMA-ALDRICH

fluid with a characteristic odour

~100

120

sparingly soluble in H₂O; soluble in most organic solvents

3000 mg/kg rat

5980 mg/kg mouse

4200 mg/kg rat

2900 mg/m³ (4 h) for rats

Antimicrobial effectiveness/applications

Fenpropimorph was created by BASF as a systematizing plant protection fungicide. The broad-spectrum microbicide may be used as an active ingredient in formulations for wood protection, e.g. for the protection of freshly cut timber. Outstanding is its efficacy against *Penicillium brevicaulis* and *Lentinus tigrinus*.

Microbicide group (substance class)

Chemical name

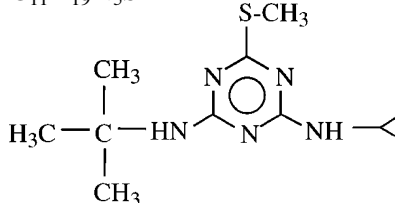
Chemical formula

Structural formula

20. VARIOUS COMPOUNDS

20.4. N-Cyclopropyl-N'-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine

C₁₁H₁₉N₅S



Molecular mass	253.36
CAS-No.	28159-98-0
EC-No.	248-872-3
EPA-Reg.	approval for antimicrobial applications
Synonym/common name	N'-tert.-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine
Supplier	INT. SPEC. PROD.
Chemical and physical properties	
Appearance	yellowish powder with a slight odour
Content (%)	96
Boiling point/range °C (101 kPa)	>200
Melting point °C	128–133
Density g/ml (20 °C)	1.1
Vapour pressure hPa (25 °C)	8.8×10^{-7}
Flash point °C	>200
Log POW at 25 °C	3.95
pH at 20 °C	7 (7 mg/l)
Stability	thermally stable under normal storage conditions
Solubility g/l (20 °C)	0.007 in H ₂ O; ~50 in xylene (100 at 40 °C), ~120 in methyl isobutyl ketone (310 at 60 °C), ~150 in butyl acetate, ~10 in propylene glycol, ~50 in octanol, ~50 in Texanol [®]

Toxicity data (Source: DEGUSSA)

LD ₅₀ oral	>2000 mg/kg rat
LD ₅₀ dermal	>2000 mg/kg rat
LC ₅₀ on inhalation	>4.1 mg/l air (4 h) for rats

In tests with rabbits non-irritant on the skin, mild irritant at the eyes. A skin sensitizer according to the guinea pig test. In tests with rats non-teratogenic and non-embryotoxic. Ames test: negative. Micronucleus test: non-genotoxic. V 79 point mutation test, DNA repair (human fibroblasts): non-mutagenic.

Ecotoxicity: The triazine derivative is extremely hazardous for water.

LD ₅₀ for Bluegill sunfish	2.9 mg/l (96 h)
for Rainbow trout	0.94 mg/l (96 h)
for Zebra fish	4.0 mg/l (96 h)
EC ₅₀ for Daphnia magna	2.4 mg/l (48 h)
EC ₅₀ for Mysid shrimps	0.40 mg/l (96 h)
EC ₅₀ for Oyster embryo-larvae	3.2 mg/l (48 h)

The input of the product into water purification plants should be avoided.

Antimicrobial effectiveness/applications

The s-triazine derivative is not effective against bacteria, yeasts or fungi. It is an inhibitor of photosynthesis and accordingly effective against algae. The activity spectrum is broad; it covers fresh water algae and sea water algae. The minimum inhibition concentrations for these organisms range between 0.01 and 0.1 mg/litre. The very low water-solubility of the compound in combination with its high anti-algal activity and favourable toxicological data have led to the use of the s-triazine derivative as an active ingredient in antifouling coatings for the substitution of ecotoxic organotin compounds (see 19.). 1–5% of the algaecide are normally incorporated in antifouling paints. The s-triazine derivative shows no effect, however, against fouling by sea animals such as barnacles, serpulids and molluscs.

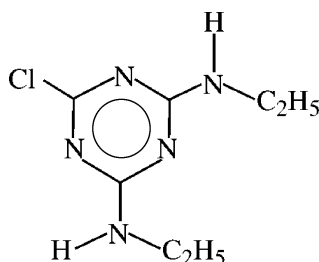
Combination with copper compounds therefore may be required.

The algaecide may also be used in paints, caulks, stucco and coatings to inhibit algae growth on corresponding exterior surfaces. Recommended use levels move between 0.03–0.5% based on total paint weight. However, to protect the surfaces also from mould growth one has to apply the algaecide together with a suitable fungicide, e.g. Chlorothalonil (17.19.), Tebuconazole (14.1.).

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

20. VARIOUS COMPOUNDS

20.5. 2,4-bis(Ethylamino)-6-chloro-1,3,5-triazine
 $C_7H_{12}ClN_5$



Molecular mass
CAS-No.
EC-No.
EPA TSCA
EPA TSCATS
Synonym/common name
Supplier

201.66
122-34-9
204-535-2
Section 8(B) Chemical Inventory
Data base, Jan. 2001
Simazine
SIGMA-ALDRICH

Chemical and physical properties

Appearance
Content (%)
Melting point °C
Vapour pressure hPa (20°C)
Flash point °C
Stability

white to pale yellow solid
approx. 100
225–227 (decomposition)
10.8
>100
heat resistant up to 200°C; hydrolyses in alkaline media to the inactive 6-hydroxy compound
sparingly soluble in H₂O; soluble in most organic solvents

Solubility

Toxicity data

LD₅₀ oral

971 mg/kg rat
>5000 mg/kg rabbit
>5000 mg/kg guinea pig
>5000 mg/kg rat
>10200 mg/kg rabbit

LD₅₀ dermal

LD₅₀ intravenous
LC₅₀ on inhalation
Irritant to skin and eyes.

100 mg/kg mouse
9800 mg/m³ (4 h) for rats

NTP carcinogenesis studies on test (two year studies), Oct. 2000.

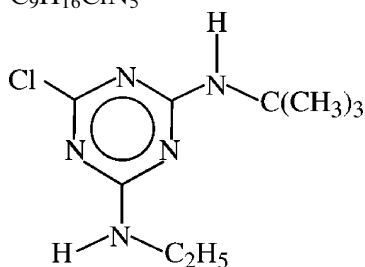
Antimicrobial effectiveness/applications

Simazine was introduced as a selective herbicide in 1959. The s-triazine derivative exhibits also algacidal efficacy. Accordingly it is applied in ponds to inhibit the growth of algae and plants. As a paint film algacide it has not gained importance.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula

20. VARIOUS COMPOUNDS

20.6. 2-tert.-Butylamino-6-chloro-4-ethylamino-s-triazine
 $C_9H_{16}ClN_5$

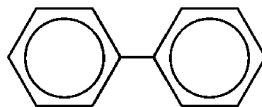


Molecular mass	229.72
CAS-No.	5915-41-3
EC-No.	227-637-9
EPA TSCATS	Data base, Jan. 2001
EPA/FIFRA	approval for the treatment of cooling and retort water recirculating towers, and ornamental fountains
Synonym/common name	2-chloro-4,6-dialkylamino-1,3,5-triazine, Terbutylethylazine
Supplier	FMC CORP., SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to pale yellow liquid
Content (%)	approx. 100
Melting point °C	177–179
Vapour pressure hPa (20°C)	0.2
Flash point °C	>100
pH in aqueous dispersion (3.8–4.2%)	6–9
Stability	stable in acid, neutral and slightly alkaline solutions; is unaffected by oxidizing biocides; stable to UV light
Solubility g/l (20°C)	0.0085 in H ₂ O; soluble in most organic solvents
Toxicity data	
LD ₅₀ oral	1845 mg/kg rat
LD ₅₀ dermal	>3000 mg/kg rat >3000 mg/kg rabbit
LC ₅₀ on inhalation	>3510 mg/m ³ (4 h) for rats
LD ₅₀ parenteral	2160 mg/kg rat
Irritant to skin, mucosa and eyes.	

Antimicrobial effectiveness/applications

As is typical for such s-triazine derivatives the compound exhibits algacidal efficacy. Aqueous dispersions containing 3.8–4.2% active ingredient have been developed to control algae in industrial water systems and ornamental fountains.

Microbicide group (substance class)	20. VARIOUS COMPOUNDS
Chemical name	20.7. Biphenyl
Chemical formula	C ₁₂ H ₁₀
Structural formula	



Molecular mass	154.20
CAS-No.	92-52-4
EC-No.	202-163-5; E230 in EEC Directives for food additives
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	phenylbenzene
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to pale yellow solid with a characteristic odour
Content (%)	≥98
Boiling point/range °C (101 kPa)	255
Melting point °C	69–72
Density g/ml (20°C)	0.992

Vapour density g/l	5.31
Flash point °C	110
Upper flammability limit % v/v i. air	5.8
Lower flammability limit % v/v i. air	0.6
Stability	extraordinary heat-resistant
Solubility	virtually insoluble in H ₂ O; highly soluble in alcohol and ether

Toxicity data

LD ₅₀ oral	2140 m/kg rat 1900 mg/kg mouse 2400 mg/kg rabbit
LD ₅₀ dermal	>5010 mg/kg rabbit
LD ₅₀ intravenous	56 mg/kg mouse

In tests with rabbits severe skin irritation after application of 500 µl (24 h), mild eye irritation after application of 100 mg. ADI-level (WHO/FAO): 0.05 mg/kg body weight/day.

Occupational exposure limits France, Germany, UK, USA: 0.2 ml/m³ (ppm)

Antimicrobial effectiveness/applications

Biphenyl is a membrane active microbicide which in particular is active against fungi. However, there are gaps in the spectrum of effectiveness. But biphenyl is able to inhibit the growth of fungi occurring during storage on citrus fruits. It is applied by a dipping treatment or by packing the fruits in packaging material impregnated with the volatile biphenyl.

Microbicide group (substance class)

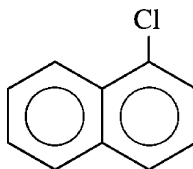
Chemical name

Chemical formula

Structural formula

20. VARIOUS COMPOUNDS

20.8. 1-Chloronaphthalene

C₁₀H₇Cl

Molecular mass

CAS-No.

EC-No.

EPA TSCATS

Synonym/common name

Supplier

162.52

90-13-1

201-967-3

Data base, Jan. 2001

alpha-chloronaphthalene

SIGMA-ALDRICH

Chemical and physical properties

Appearance

clear, slightly yellow liquid with a strong characteristic odour

Content (%)

~90 (contains approx. 10% 2-chloronaphthalene)

Boiling point/range °C (101 kPa)

259 (117–120 at 1.6 kPa)

Melting point °C

8–5

Density g/ml (20°C)

1.192

Vapour pressure hPa (20°C)

~0.05 (0.4 at 50°C)

Refractive index n_D (20°C)

1.633

Flash point °C

121

Solubility

sparingly soluble in H₂O, highly soluble in most organic solvents

Toxicity data

LD ₅₀ oral	1540 mg/kg rat 1091 mg/kg mouse
LC ₅₀ on inhalation	>20 mg/l (1 h) for rats

Toxicity data (source: Buckman) for a formulation basing on 2-(2-methoxyethoxy) propanol; MBT content: 10%

LD₅₀ oral 309.8 mg/kg rat
LD₅₀ dermal 1000 mg/kg rabbit

Toxic by inhalation of sprays or aerosols. Hazardous in case of skin contact: causes skin-inflammation and sensitization. Corrosive to eyes.

Ecotoxicity:g

Toxic to aquatic organisms.

LC₅₀ for Bluegill sunfish 2.7 mg/l (96h)

Antimicrobial effectiveness/applications

MBT is a powerful microbicide of limited stability; it is used to control bacteria, algae, yeasts and fungi in industrial water systems, especially in paper mills.

Table 146 Minimum inhibition concentrations (MIC) of MBT in nutrient agar

Test organism	MIC (mg/litre)
<i>Aspergillus niger</i>	2.5–10
<i>Aerobacter aerogenes</i>	5–10
<i>Clostridium sp.</i>	5–10
<i>Bacillus megaterium</i>	5
<i>Staphylococcus aureus</i>	5
<i>Chlorella sp.</i>	3
'Black algae' (from pools)	12

Chemical name 20.9.2. Methyl isothiocyanate
Chemical formula C₂H₃NS
Structural formula H₃C-N = C = S
Molecular mass 73.12
CAS-No. 556-61-6
EC-No. 209-132-5
EPA TSCATS Data base, Jan. 2001
Synonym/common name isothiocyanic acid methyl ester
Supplier SIGMA-ALDRICH

Chemical and physical properties

Appearance colourless crystalline powder
Content (%) approx. 100
Boiling point/range °C (101 kPa) 118 - 120
Melting point °C 35 - 36
Density g/ml (37°C) 1.07
Vapour pressure hPa (20°C) 28
Flash point °C 32
Stability heat sensitive; incompatible with alcohols, alkalis, amines, oxidizing agents

Toxicity data

LD₅₀ oral 72 mg/kg rat
90 mg/kg mouse
LD₅₀ dermal 1820 mg/kg mouse
LD₅₀ subcutaneous 50 mg/kg mouse
LD₅₀ intraperitoneal 54 mg/kg rat
82 mg/kg mouse
LC₅₀ on inhalation 1900 mg/m³ (1 h) for rats

In tests with rabbits moderate skin irritation after application of 500 mg (24 h); severe eye irritation after application of 100 mg. Methyl isothiocyanate obviously is very toxic and extremely hazardous for aquatic animals, but intrinsically degradable.

Antimicrobial effectiveness/applications

Methyl isothiocyanate can be characterized as a high reactive biocidal substance. Although it generally is not applied as such, it is described here, as the effectiveness of Dazomet (3.3.25.) bases to a large extent on the liberation of methyl isothiocyanate.

Chemical name	20.9.3. Allyl isothiocyanate
Chemical formula	C_4H_5NS
Structural formula	$H_2C = CH-CH_2-N = C = S$
Molecular mass	99.15
CAS-No.	57-06-7
EC-No.	200-309-2
EPA TSCA	Section 8 (B) Chemical Inventory
Synonym/common name	allyl mustard oil, isothiocyanic acid allyl ester
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	colourless to pale yellow liquid with a strong lachrymatory odour
Content (%)	≥ 98
Boiling point/range °C (101 kPa)	151–153
Solidification point °C	–80
Density g/ml (20°C)	1.020
Refractive index nD (20°C)	1.532
Stability	sensitive to light, air, heat, heavy metals
Solubility	hardly soluble in H_2O ; soluble in alcohol, ether, benzene
Toxicity data	
LD ₅₀ oral	112 mg/kg rat 308 mg/kg mouse
LD ₅₀ subcutaneous	92 mg/kg rat 80 mg/kg mouse
LD ₅₀ dermal	88 mg/kg rabbit
Irritation of the rabbit eye after application of 2 mg.	

Antimicrobial effectiveness/applications

Allyl isothiocyanate is a labile and reactive substance which disposes of insecticidal and microbicidal efficacy, but has not gained major importance in microbicide application fields.

Microbicide group (substance class)	20. VARIOUS COMPOUNDS
Chemical name	20.10. Sustainable active microbicidal (SAM) polymers

SAM-Polymers[®] can be regarded as contact microbicides which interact with adhering micro-organisms. Characteristic of them is a high proton density which disturbs the (proton) metabolism of microbe cells. Essential for the activity of SAM-polymers is enrichment at the surface of products to be protected against microbial growth, as incorporated SAM-polymers (e.g. in plastics) do not migrate to the surface. Their interaction with micro-organisms is not accompanied by inactivation. Hence long lasting effectiveness without endangering the environment can be guaranteed. Remarkable is that the starting products for SAM-polymers, the corresponding monomers, in general do not exhibit antimicrobial activity worth mentioning.

Chemical name	20.10.1 Poly(tert.-butylaminoethyl)methacrylate
Chemical formula:	polymer: $(C_{10}H_{19}NO_2)_n$
Structural formula:	monomer: $H_2C=C(CH_3)-COO-CH_2-CH_2-NH-C(CH_3)_3$
Molecular mass:	polymer: ~228000; monomer: 185.27
CAS-No.	monomer: 3775-90-4
EC-No.	223-228-4
EPA-Reg.	in preparation
Synonym/common name	polymethacrylic acid tert.-butylaminoethyl ester
Supplier	DEGUSSA/CREAVIS

Chemical and physical properties

Appearance	white powder, odourless
Content (%)	~100
Glass temperature °C	approx. 40
Density g/ml (20°C)	0.98
Stability	thermal degradation starts at approx. 190°C
Solubility	virtually insoluble in H_2O (<2 mg/l at 20–80°C); highly soluble (>50%) in ethanol, ethyl acetate, acetone, >20% in DOP

Toxicity data (source: CREAVIS)

LD ₅₀ oral	>2000 mg/kg rat
dermal	>2000 mg/kg rat

Not irritant to the skin (OECD Test Guideline 404); irritating to eyes (OECD Test Guideline 405).

Not a skin sensitizing agent.

Ames test, chromosomes aberration, cytogenetic test: negative

Occupational exposure limit: 6 mg/m³.

Antimicrobial effectiveness/applications

The contact microbicide exhibits efficacy against a broad spectrum of microorganisms: Gram-negative and Gram-positive bacteria, fungi, yeast, algae. Figure 26 demonstrates the reduction in viable cell counts of *Pseudomonas aeruginosa* DSM 939 in dependence on contact time in a suspension of 0.1% poly (tert.-butylaminoethyl)methacrylate.

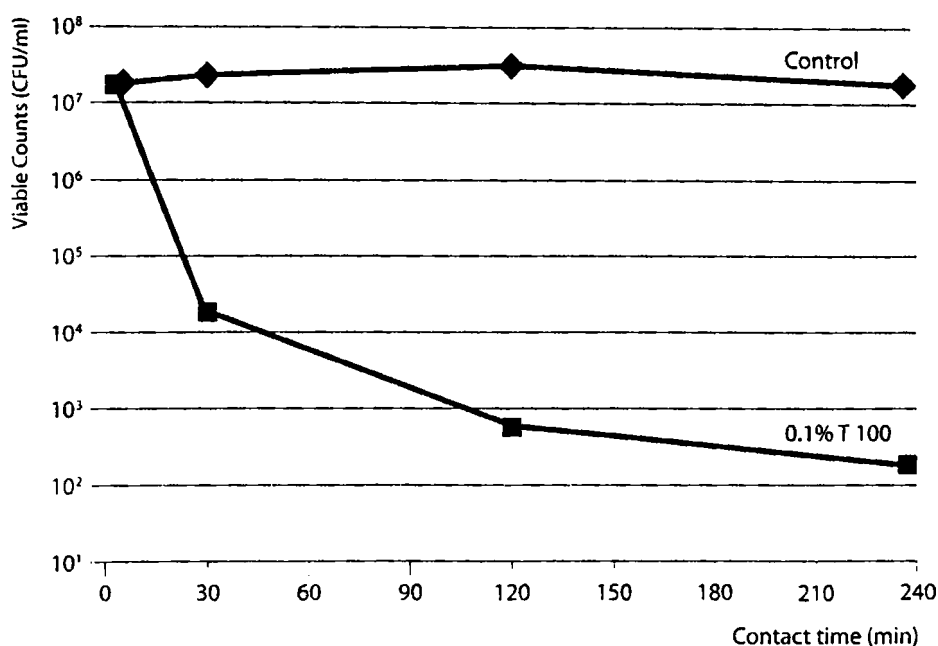


Figure 26 Reduction in viable cell counts of *Pseudomonas aeruginosa* DSM939 in dependence on contact time of SAM-Polymer 20.10. (T100). Source: CREAVIS.

The microbicidal polymer is suited as an additive for the incorporation into plastic material. More economic is the enrichment of the additive at the surface of the plastic material by the application of a corresponding coating. The fact that the microbicidal polymer is heat resistant, not volatile and not leachable opens a wide field of applications.

There exists a great variety of applicabilities for plastic materials displaying durable antimicrobial properties. This is also valid for coatings and wood treated with SAM-polymer.

20.11. Antibiotics

Antibiotics may be defined as secondary metabolites of micro-organisms. In contrary to primary metabolites (proteins, carbohydrates, nucleic acids, lipids) which play an essential role in the growth and multiplication of cells secondary metabolites are of no importance in that respect. Antibiotics dispose of a relative low molecular mass and the ability to exhibit microbistatic or microbicidal efficacy in/on other microbe species by impairing the cell wall biosynthesis, the cytoplasmic membrane, the oxidative phosphorylation. Because of there extremely high antimicrobial activity antibiotics are mainly used as chemotherapeutics; however, some antibiotics are also used in the food industry for the protection of food against deterioration; e.g. Nisin (20.11.1.), Pimaricin (20.11.2.). But these applications will be more and more restricted or even completely banned as microbes may acquire resistance which represents a severe problem in chemotherapy with antibiotics. Acquired resistance is a consequence of the selection pressure on a microbe population in the presence of microbicides. Chemotherapy with an antibiotic the application of which has led to the selection of mutant resistant organisms is no longer successful.

Microbicide group (substance class)

Chemical name

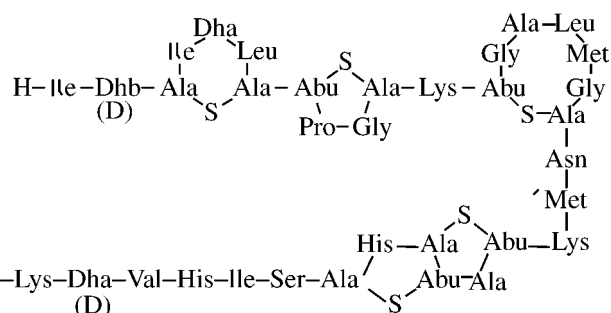
Chemical formula

Structural formula

20.11. ANTIBIOTICS

20.11.1. Nisin A

$C_{143}H_{230}N_{42}O_{37}S_7$



Abu α -Aminobuttersäure

Dha Dehydroalanin

Dhb Dehydrobutyrin

Molecular mass

CAS-No.

EC-No.

FAO/WHO

Synonym/common name

Supplier

Chemical and physical properties

Appearance

Content (%)

Stability

Solubility g/l

Toxicity data

LD₅₀ intravenous
oral

ADI-value (FAO/WHO)

3354.25

1414-45-5

215-807-5; E234 in EEC-Directives for food additives

accepted food additive

1-thia-4,7,10,13,16,19-hexaazacyclodocasane, Nisin 1-34
from *Streptococcus lactis*

SIGMA-ALDRICH

odourless white powder

normally 2.5 in a mixture of NaCl (75) and non-fat dry
milk (22.5)

extremely resistant to heat; solutions in dilute acids are
stable to boiling; at pH values < 7 activity decreases

0.075 in H₂O at pH 7; 4,2–12 in H₂O at pH 2–6

100 mg/kg mouse

6900 mg/kg mouse

0.132 mg/kg body weight/day

Antimicrobial effectiveness/applications

Nisin is a polypeptide the 34 amino acids of which form five structural units closed over sulphur bridges. The antibiotic which is produced by lactic acid bacteria has no therapeutical importance. It is primarily effective against Gram-positive bacteria including *Chlostridium botulinum* strains. Microbicidal effectiveness against strains of *Staphylococcus*, *Streptococcus*, *Bacillus chlostridium* and *Corynebacterium* is achieved by 0.018–36 ppm Nisin. As an antichlostridium agent Nisin may be applied in cheese wraps and as an heat adjunct in canned food; permissible addition rates 10–100 ppm. pH optimum 6,5–8, preferably 7. For further information see Part One, chapter 5.10. “Food and Beverage Preservation”.

Microbicide group (substance class)

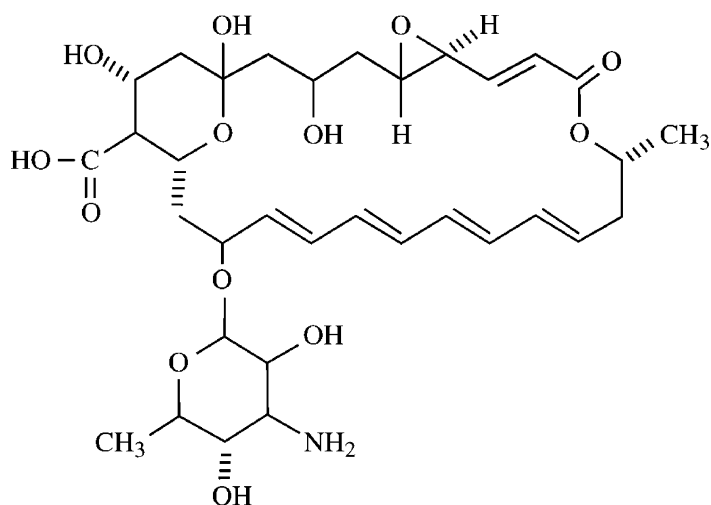
Chemical name

Chemical formula

Structural formula

20.11. ANTIBIOTICS

20.11.2. Pimaricin

 $C_{33}H_{47}NO_{13}$ 

Molecular mass

CAS-No.

EC-No.

FAO/WHO

Synonym/common name

Supplier

665.75

7681-93-8

231-683-5; E235 in EEC-Directives for food additives approved food additive

Natamycin

SIGMA-ALDRICH

Chemical and physical properties

Appearance

Content (%)

Melting point °C

Stability

Solubility

colourless crystals

normally 2.5 in aqueous suspension

200 (decomposition)

sensitive to light; limited heat resistance in cheese coatings; stable in dry state

moderately soluble in dimethyl formamide, methyl pyrrolidone, propylene glycol, diethylene glycol; hardly soluble in methanol and water

Toxicity data

LD₅₀ oral

ADI-value (FAO/WHO)

1500 mg/kg rat

0.3 mg/kg body weight/day

Antimicrobial effectiveness/applications

Pimaricin is a macrocyclic polyene antibiotic of the tetraene type with mycosamine as amino sugar glycoside. It is produced in cultures of *Streptomyces natalensis* and *Streptomyces chattanoogensis* and distinguishes by extraordinary antifungal activity; MIC values for fungi are ≤ 10 ppm. Pimaricin's mechanisms of action bases on bonding to membrane sterols thus disturbing membrane permeability. A typical application of Pimaricin as

an approved food additive is the treatment of sausages and chesse by dipping or spraying to prevent mould growth on these products. For further information see Part One, chapter 5.10 "Food and Beverage Preservation".

21. Oxidizing agents

Oxidizing agents such as hydrogen peroxide, peroxy acids, halogens and halogen releasing compounds have a microbiocidal effect thanks to their strong oxidation power which is also directed, unspecifically, towards organic matter, that is, towards micro-organisms. It is in the nature of this effect not to be selective and to cover Gram-positive and Gram-negative bacteria, yeasts, fungi and algae but also, advantageously, spores and viruses. The aggressiveness of the agents under discussion, not only directed towards microbes, must be taken into account when considering their use as microbicides and does limit their applicability. Another factor to be considered in this connection is the oxidizing agents' intrinsic limited stability – which on the other hand offers the advantage that the agents can be used without ecologically harmful residues being obtained. In consideration of their characteristics, the oxidizing agents are used on quite a large scale as sanitizers in food processing plants, public buildings, hospitals, schools, restaurants and household; another large application field is water treatment: pool and drinking water, process water, sewage and waste water effluents. Their antimicrobial efficacy is accompanied by bleaching and deodorizing effect.

Microbicide group (substance class)	21.1. PEROXY COMPOUNDS
Chemical name	21.1.1 Hydrogenperoxid
Chemical formula	H ₂ O ₂
Structural formula	H-O-O-H
Molecular mass	34.02
CAS-No.	7722-84-1
EC-No.	231-765-0
EPA TSCATS	Data base, Jan. 2001
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	clear, colourless, odourless liquid
Content (%)	≥30 in H ₂ O
Density g/ml (20°C)	1.11
Stability	decomposes slowly to H ₂ O and oxygen, if not stabilized, e.g. by sulphuric or phosphoric acid; decomposition is accelerated by traces of heavy metals, alkalis and dust
Solubility	miscible with water
Toxicity data	
LC for rats and guinea pigs: 100 ppm after inhalation.	
Corrosive to skin, mucous membranes and eyes.	
Occupational exposure limit Germany 1.4 mg/m ³ (1 ml/m ³).	

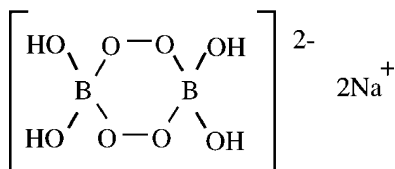
Antimicrobial effectiveness/applications

Hydrogen peroxide generates hydroxyl radicals (H-O·) which are highly reactive and responsible for the antimicrobial action. The enzymes catalase and peroxidase which are produced by respiring cells to protect the cells from damage by steady-state levels of metabolically generated hydrogen peroxide are overwhelmed by higher hydrogen peroxide concentrations, e.g. 3–6%. Such concentrations are used in disinfectants and sanitizers. They are effective within minutes and therefore not considerably disturbed by inactivation processes occurring simultaneously, e.g. consumption of active ingredient by organic matter. The number of living cells in water can be sufficiently reduced by the addition of 1% of a 3% hydrogen peroxide solution. Heavily contaminated products, e.g. water based paints and thickener solutions showing signs of viscosity loss due to enzymatic degradation may be saved by the addition of 0.05–0.1% of a 30% hydrogen peroxide solution with stirring. After 24 h, when microbes and enzymes are inactivated and most of the unpleasant odours are eliminated by oxidation, the viscosity of the material is restored with additional thickener and the in-can/in-tank protection by the incorporation of a suitable preservative. The antimicrobial activity and decomposition of hydrogen peroxide increases and

accelerates with increasing temperature. The pH for optimum efficiency is in the acidic range; in alkaline media hydrogen peroxide decomposes too quickly.

Microbicide group (substance class)
Chemical name
Chemical formula (cyclic structure)
Structural formula

21.1. PEROXY COMPOUNDS
21.1.2. Sodium perborate tetrahydrate
 $B_2H_4Na_2O_8$



Molecular mass
CAS-No.
EC-No.
Supplier

199.65
10486-00-7
239-172-9
SIGMA-ALDRICH

Chemical and physical properties

Appearance
Content (%)
Melting point °C
Density g/ml (20°C)
Stability

white, odourless powder
 ≥ 97
65
1.731
releases in aqueous solution very slowly OOH^- ions resp. oxygen, but much quicker at temperatures $\geq 60^\circ\text{C}$; sensitive to humidity and fine metal powders
moderately soluble in H_2O with alkaline reaction

Solubility

Toxicity data

LD_{50} oral

1060 mg/kg mouse
1200 mg/kg rat

LD_{50} intraperitoneal

538 mg/kg mouse

50 mg applied to the rabbit eye caused moderate irritation.

Antimicrobial effectiveness/applications

Sodium perborate is used in washing powders/detergents as bleaching and disinfecting agent. The release of oxygen at temperatures $< 60^\circ\text{C}$ can be activated by the addition of perborate activators, e.g. tetraacetythylenediamine which reacts in alkaline media with the peroxyborate under formation of peroxyacetic acid (21.1.3.) The latter is a less stable oxygen releasing compound and accordingly more active than the peroxyborate.

Microbicide group (substance class)
Chemical name
Chemical formula
Structural formula
Molecular mass
CAS-No.
EC-No.
Synonym/common name
Supplier

21.1. PEROXY COMPOUNDS
21.1.3. Peroxyacetic acid
 $\text{C}_2\text{H}_4\text{O}_3$
 $\text{H}_3\text{C}-\text{CO}-\text{OOH}$
76.05
79-21-0
201-186-8
peracetic acid
SIGMA-ALDRICH

Chemical and physical properties

Appearance
Content (%)
Density g/ml (20°C)
Vapour pressure hPa (25°C)

clear, colourless liquid with a pungent odour
 ~ 39 in acetic acid (~ 45)
1.145
26.6

Refractive index n_D (20°C)	1.391
Flash point °C	56
Stability	decomposes slowly to acetic acid (8.1.2.) and water and oxygen; in stabilized solutions there is an equilibrium between $H_3C-CO-OOH$, H_2O_2 and $H_3C-CO-OH$; sensitive to light; its instability is promoted by heavy metal ions and elevated temperatures; explosive when heated up to 110°C
Solubility	highly soluble in water, ethanol and ether
Toxicity data	
Corrosive to skin, eyes and mucous membranes. – Non mutagenic.	
For further information see acetic acid (8.1.2.) and hydrogen peroxide (21.1.1.)	

Antimicrobial effectiveness/applications

According to Eggensperger (1979) peracetic acid is several orders of magnitude more effective than hydrogen peroxide. The spectrum of activity is extraordinarily broad; it covers Gram-positive and Gram-negative bacteria, yeasts, fungi, spores and viruses. The following concentrations of peracetic acid (10% a.i.) act microbicidally on bacteria within 2 min, 5 min and 24 h respectively: 20 mg/litre, 10 mg/litre, 1 mg/litre. The sporicidal activity of peracetic acid is characterized by high rapidity. Advantageously peracetic acid is effective, that means breaks down, without leaving residues. In concentrations of 0.1–0.5% it may be used for the cold sterilization of surgical instruments. In detergents for the cold wash of heat sensitive synthetic textiles peracetic acid is used as a sanitizer. As far as ion exchange resins are not affected by peracetic acid the peracid can be used for the disinfection of resins particularly in the food industry.

The optimum pH for the microbicidal action of peracetic acid varies between 2.5 and 4.

Microbicide group (substance class)	21.1. PEROXY COMPOUNDS
Chemical name	21.1.4 Magnesium bis(2-carboxylate-monoperoxybenzoic acid) hexahydrate
Chemical formula	$C_{16}H_{10}MgO_{10}$
Structural formula	$\left[\begin{array}{c} \text{C} - \text{C} = \text{C} - \text{CO}_3\text{H} \\ \quad \\ \text{C} - \text{C} = \text{C} - \text{CO}_2^- \end{array} \right]_2 \quad \text{Mg}^{2+} \cdot 6\text{H}_2\text{O}$
Molecular mass (hexahydrate)	494.65
CAS-No.	84665-66-7
EC-No.	279-013-0
MITI Reg. (Japan)	No. 3-3807
Australia	listed in Inventory of Chem. Substances
Synonym/common name	monoperoxyphthalic acid magnesium salt hexahydrate
Supplier	LONZA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white crystalline powder with a slight odour of peracid
Content (%)	≥98 (available oxygen: 5.4; H_2O : max. 0.5)
Melting point °C	96
Bulk density kg/m^3	550–700
pH (10 g/l in H_2O) at 20°C	4.5–5.5
Stability	no decomposition below 96°C; loss of active oxygen at 20°C (after 12 month) 5%; reacts with salts of heavy metals and fine powder of heavy metals
Solubility g/l	In H_2O : 130 at 5°C, 220 at 30°C

Toxicity data (source: LONZA)

LD ₅₀ oral	>2000 mg/kg rat
LD ₅₀ dermal	>2000 mg/kg rabbit (24 h)

In tests with rabbits severe skin irritation (exposure: 24 h); corrosive to rabbit eyes. Mutagenicity (Ames) test: negative.

Ecotoxicity:

LC ₅₀ for Rainbow trout	56 mg/l (96 h)
EC ₅₀ for Daphnia magna	20–37 mg/l (48 h)

Readily degradable (100% within 28 days).

Antimicrobial effectiveness/applications

The peracid salt may be used as a general purpose disinfectant, disinfectant for hospitals, food industry and institutions. It is compatible with many nonionic, cationic, anionic and amphoteric surfactants. Recommended use concentrations for the treatment of surfaces according to tests with *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Candida albicans* (source: LONZA): 0.5% (60 min) for clean surfaces, 1.0% (60 min) for dirty surfaces

Microbicide group (substance class)

21.1. PEROXY COMPOUNDS

Chemical name

21.1.5. Higher peroxycarboxylic acids

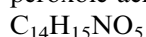
Peroxy monocarboxylic acids having a carbon chain length of 5–8 carbon atoms show in comparison to peroxyacetic acid even enhanced activity against a broad spectrum of micro-organisms including spores and also they are sufficiently soluble in water to be used in disinfectants. However, aqueous solutions of these peroxyacids have only very limited stability. A solid, substantially water insoluble organic peroxyacid, namely 1,12-diperoxydodecanedioic acid shows effective killing ability with respect to various bacteria, such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus mirabilis* and *Escherichia coli*, but fails to kill *Pseudomonas aeruginosa* within an acceptable killing time of less than 30 min at appropriate concentrations of 100 ppm active oxygen content. However, aqueous formulations of 1,12-diperoxydodecanedioic acid containing a synergistically effective amount of a sequestering agent, such as nitriloacetic acid and a surfactant plus a buffer to effect a pH of the composition in the range of from 2 to 7 exhibit a broad spectrum of effectiveness including *Pseudomonas aeruginosa* at reasonable concentrations (Ploumen, 1991).

Another peroxycarboxylic acid should be mentioned:

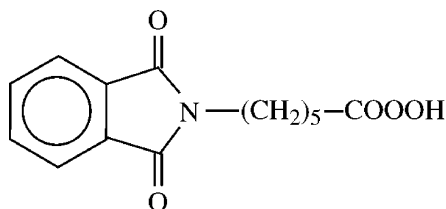
Chemical name

21.1.5.1. 6-(1,3-dioxo-1,3-dihydro-indol-2-yl)-hexaneperoxoic acid

Chemical formula



Structural formula



Molecular mass

277.28

CAS-No.

128275-31-0

EC-Notification

[N 082] A (checked and found acceptable)

Synonym/common name

 ϵ -phthaloylaminoperoxycaproic acid (PAP)

Supplier

AUSIMONT, HENKEL

Chemical and physical properties

Appearance

solid

Content of active oxygen (%)

5.8

Density g/ml (20°C)

1.364

pKa value

7.90 $\pm$ 0.2

Solubility

sparingly soluble in water; soluble in alkaline media

Antimicrobial effectiveness/applications

PAP disposes of decolouring and antibacterial properties even at low temperatures. pH optimum: 4–11.

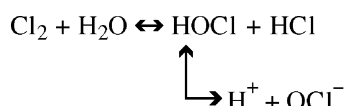
Microbicide group (substance class)

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

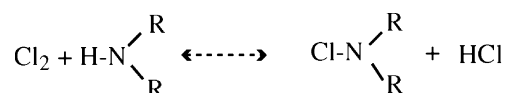
Halogens and hypohalogenites are strong oxidizing agents and consequently exhibit microbicidal efficacy. Among the halogens, iodine, chlorine and bromine are of practical significance as microbicides. Fluorine is not appropriate for practical applications as it is far too toxic, irritant and corrosive. The most important product is chlorine.

Although the industrial production of chlorine and sodium and calcium hypochlorites already started in 1785, it was not before the first half of the 19th century that the deodorizing and disinfecting properties of chlorine and hypochlorites were detected and led to the use of chloride of lime in hospital wards. Before that time chlorine and hypochlorites were used in textile bleaching. In 1881 Robert Koch demonstrated scientifically the microbicidal effect of hypochlorites by exposing pure cultures of bacteria to chlorine compounds. Thirteen years later Traube proved that hypochlorites can be used successfully for the purification and disinfection of water.

The oxidation potential of halogens is a consequence of their strong affinity to electrons. The introduction of chlorine into water leads to the following equilibrium:



without loss of activity. In the presence of ammonia or other nitrogenous compounds chlorine forms chlorine releasing compounds, so-called chloramines or N-chloro compounds according to the following equation:



From the fact that the microbicidal activity of chlorine and chlorine releasing agents decreases with increasing pH one concludes that the undissociated hypochlorous acid (HOCl) and not the hypochlorite anion (OCl^-) is essential for the effectiveness. With regard to the mechanism of activity of chlorine or hypochlorous acid or N-chloramines the experiments of Friberg (1956) using radioactive ^{35}Cl and in 1957 using radioactive ^{32}P are especially informative. In view of the results of these experiments it is concluded that contact oxidation reactions of chlorine/hypochlorous acid at the bacterial cell wall cause destructive permeability changes which lead to the kill of the microbial cell before chlorine or N-chloramines accumulate in the microbe cell.

Le Chevallier et al. (1988) found that biofilm bacteria grown on the surfaces of granular activated carbon particles, metal coupons, or glass microscope slides were 150 to more than 3000 times more resistant to hypochlorous acid (free chlorine, pH 7.0) than the unattached cells. On the other hand, resistance of biofilm bacteria to monochloramine ($\text{Cl}-\text{NH}_2$) disinfection ranged from 2–100-fold more than that of unattached cells. The results suggested that, relative to the inactivation of unattached bacteria, monochloramine was better able to penetrate and kill biofilm bacteria than free chlorine. For free chlorine, the data indicated that transport of the active ingredient into the biofilm was a major rate-limiting factor. Because of this phenomenon, increasing the level of free chlorine did not increase disinfection efficiency. See also Part One, Chapter 5.1.

The greater penetrating power of mono-chloramine apparently compensated for its limited disinfection activity.

An increase in temperature increases the microbicidal activity of chlorine, hypochlorites and N-chloramines significantly, but also the consumption of the active ingredients by organic matter such as amino acids, proteins, peptones, body fluids, tissues and vegetable matter when present in a sanitizing solution. By competitive reactions chlorine is withdrawn from microbicidal action unless chlorine releasing compounds, e.g. chloramines, are formed. Sugar and starches apparently do not significantly affect the activity of chlorine nor do, according to Shere (1948), 500 ppm of alkyl aryl sulphonate.

Micro-organisms exhibit different resistances to chlorine and hypochlorites. Vegetative cells may be killed by 0.15–0.25 ppm available chlorine. Gram-positive species are less resistant than Gram-negative species and the spore forming organisms are about 10–1000 times more resistant to chlorine than the vegetative forms. For inactivation of mould spores one needs approximately 150–500 mg hypochlorite/ml. Algae are sensitive to halogens and halogen releasing compounds, too. Growth control can be achieved by 2 mg active chlorine/ml. In swimming pools treated with chlorine problems may occur by the selection of so-called “black algae” which are resistant to the active chlorine concentrations tolerated in pools.

Chemical name	21.2.1. Chlorine
Chemical formula	Cl ₂
Structural formula	Cl-Cl
Molecular mass	70.91
CAS-No.	7782-50-5
EC-No.	231-959-5
EPA TSCATS	Data base, Jan. 2001
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	heavy gas of greenish-yellow colouration with a characteristic irritating and penetrative odour
Content (%)	≥99.8
Boiling point/range °C (101 kPa)	−34
Melting point °C	−101
Vapour density g/l (20°C)	2.48
Vapour pressure hPa (20°C)	6.38
Flash point °C	not inflammable
Stability	chlorine in solution is degraded by UV light; copper, nickel, or cobalt catalyze the decomposition; stability is favoured by high alkalinity, low temperatures and absence of organic matter
Solubility g/l (in H ₂ O)	9.5 at 10°C; 4.5 at 40°C; in aqueous solution an equilibrium according the following equation adjusts: Cl ₂ + H ₂ O ↔ HOCl + HCl
Toxicity data	
LC ₅₀ on inhalation	293 mg/l (1 h) for rats 137 mg/l (1 h) for mice
Highly toxic and corrosive to skin, mucous membranes and eyes.	
Occupational exposure limits ml/m ³ (mg/m ³)	
Germany/UK	0.5 (1.5)
US TLV –TWA (ACGIH)	0.5
Ecotoxicity: Highly hazardous for aquatic animals.	

Antimicrobial effectiveness/applications

The antimicrobial activity of chlorine covers all species of micro-organisms including spores and viruses. Among the halogens chlorine exhibits the strongest sporicidal efficiency which is, however, slow. Chlorine is used for water treatment, including sewage and waste water. After treatment with chlorine the detectable concentration of active chlorine in drinking water should be ≥1 ppm; in treated swimming pool water 0.3–0.6 ppm active chlorine should be detectable. Using chlorine solutions, e.g. for disinfecting purposes, one has unconditionally to take into account the chlorine consumption by organic matter. Chlorination of waste water treatment plant effluents and other waters, such as cooling waters and industrial wastes can produce stable chlorine-containing organic compounds which may, if discharged to rivers and lakes, have biotoxic effects at chronic, low level concentrations and may contribute to such phenomena as fish population and species changes that occur below the discharges of chlorinated waste water treatment plant effluents. Jolley (1975) detected stable chlorine-containing organic substances in effluents that had been chlorinated to a 1-2 mg/litre chlorine (Cl₂) residual. The total chlorination yield is reported to be approximately 1% of the chlorine dose; approximately 99% is apparently used in oxidation reactions and converted to chloride.

Microbicide group (substance class)	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical name	21.2.2a. Sodium hypochlorite solution
Chemical formula	ClNaO
Structural formula	NaOCl

Molecular mass	74.44
CAS-No.	7681-52-9
EC-No.	231-668-3
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	Eau de Labarraque
Supplier	SIGMA-ALDRICH

Chemical and physical properties

Appearance	yellowish liquid with the characteristic odour of free hypochlorous acid
Content (%) of active chlorine	≥ 13
Boiling point °C (101 kPa)	111
Solidification point °C	-30 – -20
Density g/ml (20°C)	1.25
Vapour pressure hPa (20°C)	23.28
Stability	relatively stable in alkaline solution; unstable at lower pH values; sensitive to light; not heat resistant (to be stored at temperatures < 15°C)
Solubility	Soluble in water in any portion
Toxicity data	
LD ₅₀ oral	5800 mg/kg mouse
Corrosive to skin and mucous membranes.	
10 mg applied to the rabbit eye cause moderate irritation.	

Antimicrobial effectiveness/applications

Sodium hypochlorite solution is appropriate to control a wide spectrum of micro-organisms. The active ingredient is HOCl which increases if the pH is reduced below 7. Sodium hypochlorite therefore is especially active in neutral or slightly acidic media. It does not leave residuals, is virtually colourless, non-staining, easy to handle and most economical in use. Hypochlorite solutions are proven sanitizers used in household, public buildings, the food industry and hospitals. Water treatment is another application field for sodium hypochlorite. Quantities corresponding to 0.5 ppm active chlorine are sufficient for the treatment of Swimming pools.

Chemical name	21.2.2b. Calcium hypochlorite dihydrate
Chemical formula (a.i.)	CaCl_2O_2
Structural formula	$\text{Ca}(\text{OCl})_2 \cdot 2\text{H}_2\text{O}$
Molecular mass (a.i.)	142.98
CAS-No.	7778-54-3
EC-No.	231-908-7
EPA Reg.	approval for a wide ranges of uses
Synonym/common name	Calhypo
Supplier	ARCH

Chemical and physical properties

Appearance	white free flowing granules with a chlorine like odour
Content (%) available chlorine	≥ 65 ; (H_2O content: 5.5–8.5)
Bulk density g/ml (20°C)	min 0.8
Density g/ml of a 5% sol. i. H_2O	1.06
pH (1% solution at 25°C)	10.4–10.8
Stability	not heat resistant (to be stored at temperatures < 32°C); decomposition temperature 170–180°C; reacts under decomposition with organic materials
Solubility g/l	180 in H_2O
Toxicity data (source: ARCH)	
LD ₅₀ oral	850 mg/kg rat

LD ₅₀ dermal	>2000 mg/kg rabbit
LC ₅₀ on inhalation	approx. 1300 mg/cm ³ (1 h) for rats based on acute toxicity for chlorine (21.2.1.)

Corrosive to skin, mucous membranes and eyes.

Not known to be a reproductive or development toxin or to be carcinogenic. – Mutagenicity test (dominant lethal assay): negative.

Ecotoxicity:

LC ₅₀ for Bluegill sunfish	0.088 mg/l (96 h)
for Rainbow trout	0.16 mg/l (96 h)
EC ₅₀ for Daphnia magna	0.11 mg/l (48 h)

Antimicrobial effectiveness/applications

Calcium hypochlorite is a dry chlorine donor produced by the introduction of chlorine in aqueous suspensions of calcium oxide at –20°C. The product is suitable for use in applications where the disinfecting and oxidizing power of chlorine are needed e.g. in the beverage and food industry, and in hospitals for hard surface cleaning, for water treatment including waste water and sewage effluent, in pulp and paper mills, in tanneries.

Microbicide group (substance class)	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical name	21.2.3. Hypobromous acid
Chemical formula	BrHO
Structural formula	HOBr
Molecular mass	96.92
CAS-No.	13517-11-8
EC-No.	Unknown
Chemical and physical properties	

Hypobromous acid is an unstable intermediate which presents a strong oxidizing and bleaching agent. Enrichment is only possible up to max. 6% in aqueous solution. Salts of hypobromous acid (hypobromites), e.g. NaOBr (CAS-no. 13824-96-9), KBr (CAS-no. 13824-97-0), are obtained by introducing bromine into sodium or potassium hydroxide.

Hypobromous acid is produced by reacting sodium bromide (21.2.3a.) with hypochlorous acid [HOCl, either from sodium hypochlorite (21.2.2a.) or chlorine (21.2.1.)]. For the generation of HOBr one can proceed as follows: 0.55 g of NaBr are mixed with 5 ml of a 5.25% NaOCl solution and diluted to 100 ml with distilled water. At this ratio only HOBr will be produced.

Antimicrobial effectiveness/applications

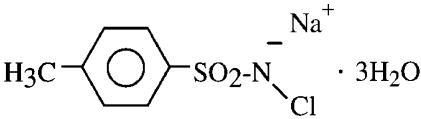
HOBr and HOCl are equal in microbicidal activity. However, since HOBr dissociates at a higher pH range than HOCl, HOBr is often used in place of HOCl in water systems operated at pH values >7, e.g. pH 8.5. Another benefit of the application of HOBr instead of HOCl are reduced corrosion rates. In the presence of amino groups containing substances HOBr forms (as does HOCl) N-bromo-amines or amides which are more effective than corresponding chlorine releasing chloramines. A disadvantage of HOBr is that it is very susceptible to oxidant demand.

Chemical name	21.2.3a. Sodium bromide
Chemical formula	BrNa
Structural formula	Na ⁺ Br [–]
Molecular mass	102.91
CAS-No.	7647-15-6
EC-No.	231-599-9
EPA TSCATS	Data base, Jan. 2001

Synonym/common name	bromide salt of sodium
Supplier	BIOLAB, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white crystals
Content (%)	≥99.5
Boiling point/range °C (101 kPa)	1390
Melting point °C	755 (dehydrate: 51)
Density g/ml (20°C)	1.0
Vapour pressure hPa (806°C)	1.33
pH (5% in H ₂ O) at 20°C)	5–7
Stability	sensitive to strong acids
Solubility	soluble in H ₂ O, ethanol and methanol
Toxicity data	
LD ₅₀ oral	3500 mg/kg rat 7000 mg/kg mouse
LD ₅₀ subcutaneous	2900 mg/kg rat 5020 mg/kg mouse
LD ₅₀ intraperitoneal	5000 mg/kg mouse
Microbicide group (substance class)	
Chemical name	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical formula	21.2.4. Chlorine dioxide
Molecular mass	ClO ₂
CAS-No.	67.46
EC-No.	10049-04-4
	233-162-8
Chemical and physical properties	
Appearance	yellowish-reddish gas, brown to red fluid, or explosive red crystals with an odour similar to chlorine
Method of manufacture	e.g. ex sodium chlorite and chlorine: $2\text{NaClO}_2 + \text{Cl}_2 \rightarrow 2\text{NaCl} + 2\text{ClO}_2$
Content (%)	~100
Boiling point/range °C (101 kPa)	10–11
Solidification point °C	–59
Density g/ml (20°C)	1.62; gas: 3.09 g/l
Stability	chlorine dioxide is an unstable gas which explodes at temperatures > –40°C; ClO ₂ /air mixtures may be explosive also as soon as the ClO ₂ concentration exceeds 10 vol %; in aqueous solutions of ClO ₂ the concentration should be limited to 6 g/l to be on the safe side
Solubility	soluble in water, miscible with lower chlorohydrocarbons
Toxicity data	
Corrosive to skin, mucosa and eyes. Occupational exposure limit: 0.1 ml/m ³ ; 0.3 mg/m ³	

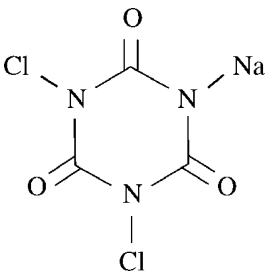
Antimicrobial effectiveness/applications

Because of the a. m. instability and risks ClO₂ has always to be prepared shortly before use. It can for example be generated from a 7.5% sodium chlorate (NaClO₃) solution after the addition of hydrochloric acid (9.5%). Chlorine dioxide's oxidation potential is 2.5 times higher than that of chlorine; therefore it is correspondingly stronger in microbicidal activity. ClO₂ disposes additionally of bleaching and deodorizing properties. It is used for the antimicrobial treatment of water, the disinfection of pipelines, as a slimicide and bleaching agent in the pulp and paper industry, for a deodorizing treatment of sewage plant effluent.

Microbicide group (substance class)	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical name	21.2.5. Chloramine-T
Chemical formula	$C_7H_7ClNNaO_2S \cdot 3H_2O$
Structural formula	
Molecular mass	227.66; trihydrate: 281.71
CAS-No.	127-65-1
EC-No.	204-854-7
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	N-chloro-4-methylbenzenesulphonamide sodium salt, sodium p-toluenesulphonylchloramide
Chemical and physical properties	
Appearance	white powder with a slight odour of chlorine
Content (%) trihydrate	Min. 99 (Cl content: 12.6)
Melting point °C	167–170 (decomposition)
pH (5% in H ₂ O) at 20°C	8–10
Stability	the chlorine liberating agent decomposes if dissolved in ethanol; explodes when heated at approximately 175°C
Solubility	soluble in H ₂ O; sparingly soluble in trichloromethane; virtually insoluble in ether
Toxicity data	
Chloramine-T is of low toxicity, but irritant to skin and mucosa though less irritating than chlorine (21.2.1.).	

Antimicrobial effectiveness/applications

The microbicidal activity of Chloramine-T bases on the release of chlorine, but the action of Chloramine-T is slower than that of chlorine itself, especially in alkaline media. Therefore it is used as an active ingredient in disinfectants and sanitizers which are applied at low pH levels and long exposure.

Microbicide group (substance class)	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical name	21.2.6. Dichloroisocyanuric acid sodium salt dihydrate
Chemical formula	$C_3Cl_2N_3NaO_3 \cdot 2H_2O$
Structural formula	
Molecular mass (dihydrate)	255.99
CAS-No.	51580-86-0
EC-No.	220-767-7
EPA TSCATS	Data base, Jan. 2001
Synonym/common name	sodium dichloro-s-triazine-2,4,6(1H, 3H, 5H)-trione, Dimanin C
Supplier	BAYER, SIGMA-ALDRICH

Chemical and physical properties

Appearance	white crystalline powder with the odour of chlorine
Content (%)	≥98 (Cl content: 27.7)
Melting point °C	240–250 (decomposition)
pH (1% in H ₂ O)	~6
Stability	sensitive to humidity
Solubility g/l (25°C)	250 in H ₂ O, ~5 in acetone

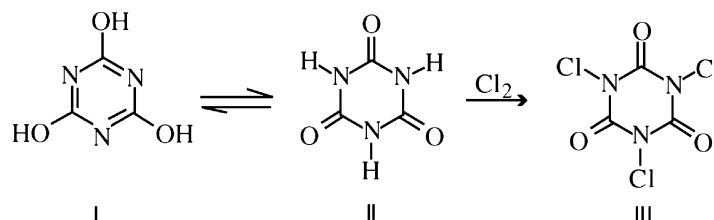
Toxicity data

LD ₅₀ oral	1420 mg/kg rat

Severely irritant to skin, mucosa and eyes. The toxicity of chloroisocyanuric acid derivatives bases on the release of active chlorine; the remaining isocyanuric acid can be regarded as more or less non-toxic; LD₅₀ oral > 5000 mg/kg rabbit.

Antimicrobial effectiveness/applications

The starting product for the manufacture of N-chloro isocyanuric acid derivatives (III) is isocyanuric acid (II) which is in a tautomeric equilibrium with cyanuric acid (I).



In comparison to other solid chlorine releasing compounds, e.g. Chloramine T (21.2.5.), N-chloroisocyanuric acid derivatives liberate a higher amount of active chlorine (see Table 147). Additionally they are more stable and easily soluble in water, in particular the alkali salts of dichloroisocyanuric acid. The simple production process for the starting product isocyanuric acid (heating of urea (H₂N-CO-NH₂) to 200–300°C) is an economical advantage. As a result chlorinated isocyanuric acid derivatives have gained significant importance as disinfecting and bleaching agents, preferably for the application in recreational waters.

The pH optimum for antimicrobial effectiveness of sodium dichloroisocyanurate is 6–10.

Table 147 Amount of active chlorine (g/kg) in some chlorine releasing compounds

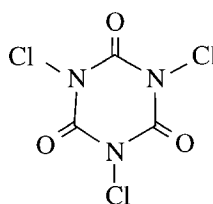
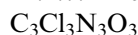
Chloramine T (21.2.5.)	240–250
Dichlorodimethylhydantoin (21.2.10.)	360
Sodium dichloroisocyanurate	600
Trichloroisocyanuric acid (21.2.7)	850

Microbicide group (substance class)

Chemical name
Chemical formula
Structural formula

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

21.2.7. Trichloroisocyanuric acid



Molecular mass
CAS-No.
EC-No.

232.41
87-90-1
201-782-8

EPA TSCATS

Synonym/common name

Supplier

Data base, Jan. 2001

trichloro-s-triazine-2,4,6(1H, 3H, 5H)-trione

SIGMA-ALDRICH

Chemical and physical properties

Appearance

white crystalline powder with the odour of chlorine

Content (%)

≥95 (Cl content: 46.8)

Melting point °C

249–251 (decomposition)

pH (1% in H₂O)

2.7–2.9

Stability

sensitive to humidity; hydrolyzes in water to HOCl and cyanuric acid

Solubility g/l (25°C)

12 in H₂O; 36 in benzene, 360 in acetone at 30°C; highly soluble in dichloroethane

Toxicity data

LD₅₀ oral

406 mg/kg rat

Severely irritant to skin, mucosa and eyes.

Antimicrobial effectiveness/applications

As the antimicrobial activity of trichloroisocyanuric acids bases on the liberation of chlorine, the efficacy of the microbicide comprises bacteria, yeasts, fungi and algae. pH optimum: 6–9.5.

Microbicide group (substance class)

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

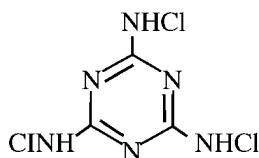
Chemical name

21.2.8. Trichloromelamine (TCM)

Chemical formula

C₃H₃Cl₃N₆

Structural formula



Molecular mass

229.46

CAS-No.

7673-09-8

EC-No.

231-648-4

Synonym/common name

2,4,6-tris(chloroamine)triazine, N,N',N''-trichloro-2,4,6-triamino-1,3,5-s-triazine

Supplier

SIGMA-ALDRICH

Chemical and physical properties

Appearance

White to pale yellow powder

Content (%)

≥98 (Cl-content: ~46)

Melting point °C

(≥300°C)

Stability

stable under normal conditions

Solubility g/l

0.34 in H₂O; soluble in acetone

Toxicity data

LD₅₀ oral

490 mg/kg mouse

Irritant to skin, mucous membranes and mucosa.

Antimicrobial effectiveness/applications

TCM is used as an active ingredient in disinfecting and bleaching preparations, although the low water solubility of TCM makes formulating difficult. A normal formulation contains 20% TCM, 68% prim. sodiumphosphate and 12% anionic detergent; pH neutral to slightly acidic.

Microbicide group (substance class)

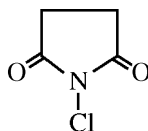
Chemical name

Chemical formula

Structural formula

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

21.2.9. N-Chlorosuccinimide (NCS)



Molecular mass

133.54

CAS-No.

128-09-6

EC-No.

204-878-8

EPA TSCA

Section 8(B) Chemical Inventory

Synonym/common name

1-chloro-2,5-pyrrolidinedione, succinchlorimide

Supplier

SIGMA-ALDRICH

Chemical and physical properties

Appearance

colourless powder

Content (%)

≥96 (Cl content: ~26)

Melting point °C

148–150

Density g/ml (20°C)

1.65

Stability

sensitive to humidity and light; incompatible with reducing agents, amines and ammonium salts

Solubility

hardly soluble in H₂O and chlorohydrocarbons; soluble and reactive in most organic solvents; soluble and inert in benzene

Toxicity data

Corrosive to skin, mucous membranes and eyes. Equivocal tumorigenic agent by RTECS criteria.

Toxic to aquatic organisms.

Antimicrobial effectiveness/applications

As a chlorine liberating compound NCS may be used for the disinfection of drinking water and as a bleaching agent for textiles. However, in the meantime it has been widely substituted by chloroisocyanuric acids, e.g. 21.2.7.

Microbicide group (substance class)

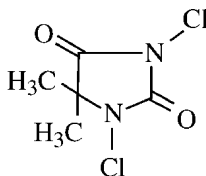
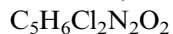
Chemical name

Chemical formula

Structural formula

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

21.2.10. 1,3-Dichloro-5,5-dimethylhydantoin (DCDMH)



Molecular mass

197.02

CAS-No.

118-52-5

EC-No.

204-258-7

EPA Reg.

Approval for antimicrobial applications

Synonym/common name

1,3-dichloro-5,5-dimethylimidazolidine-2,4-dione, Dantoin

Supplier

LONZA, SIGMA-ALDRICH

Chemical and physical properties

Appearance

white powder with a weakly stringent odour

Content (%)

≥98 (Cl content: ~36)

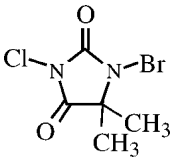
Melting point °C

132–134

pH (1% slurry in H ₂ O)	~3.5 at 25°C
Stability	sensitive to oxidizing agents and humidity; reactive in polar organic solvents
Solubility g/l (25°C)	approx. 5 in H ₂ O; soluble in methylenechloride, chloroform, ethylenechloride
Toxicity data	
LD ₅₀ oral	542 mg/kg rat 1520 mg/kg rabbit 1350 mg/kg guinea pig
LD ₅₀ dermal	>20 g/kg rabbit
In tests with rabbits severely irritant to the skin after application of 500 mg (24 h). 100 mg (24 h) applied to rabbit eyes caused severe irritation. Occupational exposure limits (mg/m ³):	
USA, ACGIH, TLV-TWA	0.2
Australia, France, The Netherlands, UK	0.2
Ecotoxicity (source: LONZA)	
Biologically well degradable (EPA test method, 42 days). Not biocaccumulative (OECD test method, 42 days with Bluegill sunfish).	

Antimicrobial effectiveness/applications

The efficacy of DCDMH at very low concentrations and the breadth of its spectrum of effectiveness bases on the release of chlorine (21.2.1.). It is used for the treatment of industrial cooling water, recreational waters, as a slimeicide for pulp and paper production, in air scrubbers and similar applications.

Microbicide group (substance class)	21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS
Chemical name	21.2.11. 1-Bromo-3-chloro-5,5-dimethylhydantoin (BCDMH)
Chemical formula	C ₅ H ₆ BrClN ₂ O ₂
Structural formula	
Molecular mass	241.47
CAS-No.	16079-88-2
EC-No.	240-230-0
EPA Reg.	approval for antimicrobial applications
Synonym/common name	1-bromo-3-chloro-5,5-dimethyl-2,4-imidazolidinedione
Supplier	BIOLAB, HOUGHTON, LONZA, SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white to yellow crystals with a faint halogen odour
Content (%)	≥97
Melting point °C	159–163
pH (aqueous suspension)	6.8
Stability	sensitive to heat, moisture, high humidity; incompatible with organic and other readily oxidizable materials and reducing agents;
Solubility	with regard to the application as a microbicide sufficiently soluble in water.
Toxicity data (source: LONZA)	
LD ₅₀ oral	485 mg/kg rat

LD₅₀ dermal > 2000 mg/kg rat

Corrosive to skin, eyes and mucous membranes.

Skin sensitization according to the guinea pig test.

Ecotoxicity:

LC₅₀ for Bluegill sunfish 1.17 mg/l (96 h)

for Rainbow trout 0.65 mg/l (96 h)

Antimicrobial effectiveness/applications

BCDMH presents a broad spectrum microbicide for water treatment applications: control of algae, bacteria and fungal slime.

Microbicide group (substance class)

21.2. HALOGENS, HYPOHALOGENITES AND HALOGEN RELEASING COMPOUNDS

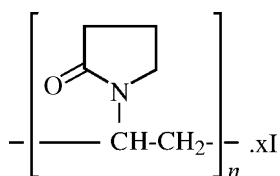
Chemical name

21.2.12. Poly(vinyl-pyrrolidone) iodine

Chemical formula

(C₆H₆NO)_n·xI

Structural formula



Molecular mass

~25000

CAS-No.

25655-41-8

EC-Notification-no.

234

EPA TACATS

Data base, Jan. 2001

Synonym/common name

Polyvidone-Iodine, PVP-iodine

Supplier

BASF

Chemical and physical properties

Appearance

yellow-brown powder

Content (%)

9-12 freely usable iodine

Bulk density g/ml (20°C)

0.43

Viscosity mPa·s (20°C)

7 (10% in H₂O)

pH (10% in H₂O)

1.5–2.5

Stability

sensitive to light and air; iodine vapour is liberated at 25°C

Solubility

soluble in water and ethanol; virtually insoluble in acetone, ether, trichloromethane

Toxicity data

LD₅₀ oral

6000 mg/kg rat

A 1% solution is slightly (reversibly) irritant to skin and eyes. The iodine bound in the complex is less corrosive and toxic than free iodine. The carrier material poly (1-vinyl-2-pyrrolidone) polymer presents favourable toxicity data, too:

LD₅₀ oral

100 g/kg rat or guinea pig

LD₅₀ intraperitoneal

12 g/kg mouse

Occupational exposure limit for iodine: 1 mg/m³.

Antimicrobial effectiveness/applications

PVP-iodine is an iodophor, in other words tamed iodine. That means the disadvantages of free iodine (i.e. unpleasant odour, skin irritations, staining of tissue and corrosion of metal surfaces) are diminished. On dilution in water the complex releases, I₂, HOI, OI[−] and I₃ which are responsible for the antimicrobial activity of PVP-iodine. However, iodine is by far not as reactive as the remaining halogens.

The disinfecting effect of PVP-iodine extends to different classes of micro-organisms including spores and viruses. The most important application is the medical disinfection of skin with solutions containing 1–10% PVP-iodine.

Microbicide group (substance class)	21. OXIDIZING AGENTS
Chemical name	21.3. Sodium iodate
Chemical formula	INaO ₃
Structural formula	NaIO ₃
Molecular mass	197.90
CAS-No.	7681-55-2
EC-No.	231-672-5. EEC-no. 10
EPA TSCA	Section 8 (B) Chemical Inventory
Synonym/common name	iodic acid sodium salt
Supplier	SIGMA-ALDRICH
Chemical and physical properties	
Appearance	white powder
Content (%)	≥99.5
Density g/ml (20°C)	4.28
pH (0.1 M in H ₂ O) at 25°C	5.5–7.0
Stability	iodates release oxygen on heating; sensitive to light and humidity; incompatible with metal powder
Solubility g/l (20°C)	90.9 in H ₂ O; insoluble in methanol
Toxicity data	
LD ₅₀ oral	505 mg/kg mouse
LD ₅₀ intraperitoneal	119 mg/kg mouse
LD ₅₀ intravenous	108 mg/kg mouse
ACGIH TLV-CL (threshold limit for iodine); USA: 0.1 ppm	

Antimicrobial effectiveness/applications

As an oxidizing agent sodium iodate disposes of a broad spectrum of antimicrobial efficacy. Since its oxidizing power is by far not as strong as that of chlorates it may be used as a preservative for cosmetic and pharmaceutical products. In the EEC Cosmetics Directive it is listed among the preservatives allowed for use in cosmetics with a maximum concentration of 0.1% for rinse-off products only. Other application: Antiseptic for mucous membranes.

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Index

- 1,2-benzisothiazolin-3-one (BIT), 239, 245, 259, 323
- 1,3-bromo-chloro-dimethylhydantoin (BCDMH), 143
- 1,3-dibromo-dimethylhydantoin (DBDMH), 143
- 1,3,2-Bezodithiazoles, 31
- 1,3,2-Bezodithiazole-S-oxides, 31
- 1,6-dihydroxy-2,5-dioxahexane, 357
- 2-benzimidazolyl-methylcaramate (BCM), 323
- 2-bromo-2-nitropropane-1,3-diol (Bronopol), 130, 243, 259
- 2-Hydroxybiphenyl, 301
- 2-mercaptopyridine, 12
- 2-methyl-4-isothiazoline-3-one (MIT), 130, 230, 245, 259
- 2-methyl-4,5-trimethylene-isothiazolin-3-one, 18
- 2-n-octyl-4-isothiazolin-3-one (OIT), 323
- 2-(thiocyanomethylthio) benzothiazole, 130, 321, 323, 435
- 2,2-dibromo-3-nitrilopionamide (DBNPA), 163, 260
- 2,4-Hexadienoic acid, 289
- 2-(4-Thiazolyl)benzimidazole, 300
- 3-Acetyl-4-hydroxy-6-methyl-pyron, 299
- 3-Aryl-5,6-dihydro-1,4,2-oxathiazines, 28
- 3-hydroxy-3-methylamino-thioacrylchloride, 19
- 3-iodo-2-propinyl-butylcarbamate (IPBC), 277, 435
- 3-mercapto-3-chloro-N-methyl-acrylamide, 19
- 3,5-Dimethyl-tetrahydro-1,3,5-2H-thiadiazine-2-thione (DAZOMET), 260
- 4-chloro-2-hydroxybiphenyl, 559
- 4-chloro-2-phenylphenol, 559
- 4-chloro-3-methylphenol (PCMC), 323
- 4-hydroxybenzoic acid ethyl ester, 294
- 4-hydroxybenzoic acid methyl ester, 294
- 4-hydroxybenzoic acid propyl ester, 294
- 4-(2-tert.-Butyl-5-methylphenoxy)phenol, 33
- 4,5-dichloro-2-(n-octyl)-isothiazolin-3-one, 19
- 4,5-trimethylene-2-methyl-isothiazolinone, 32
- 5-chloro-2-methyl-isothiazolin-3-one (CMI), 19, 130
- 5-chloro-2-methyl-4-isothiazolin-3-one (CIT), 230, 259
- 16s rDNA, 254
- 28th ATP, 245
- 76/768 EEC, 268
- β -propiolactone, 20
- abiotic factors, 97
- acceptance criteria, 279
- Achromobacter, 352
- acid producing bacteria, 161
- Acidum benzoicum, 288
- acne, 264
- acquired resistance, 20, 98
- acrolein, 162
- action of enzymes on plastics, 333
- activated halogen atoms, 16
- activated N-S bond, 16
- activated S-N bond, 31
- activated surfaces, 113
- adaptation, 98, 266, 278
- adequate protection, 278
- Aerobacter*, 319
- aerobic growth, 253
- Alcaligenes, 352
- alcohols, 20, 271
- aldehydes, 14, 20, 31, 99, 260
- algae, 123, 124
- algicides, 3
- alkaline earth ions, 278
- alkanolamides, 264
- alkylamidobetain, 272
- alkylamidoglycinate, 272
- alkylaminoethyl sulphates, 271
- alkyl ammonium compounds, 434
- alkylating agents, 20
- alkylbenzyl dimethyl ammonium salts, 272
- Alkyl dimethyl benzyl ammonium chloride, 130, 153
- alkylpolyglycocides (APG), 272
- allergenic potential, 265
- allergic to fragrances, 273
- allergy, 273
- Alternaria* sp., 361, 363
- amphipathetic molecule, 10
- amphoteric surfactants, 272
- amylases, 230
- Anabaena, 364
- anaerobic bacteria, 380
- anaerobic growth, 253
- anionic surfactants, 222, 264, 271
- antibacterial agents, 264, 265
- antibacterial efficacy, 265
- antibacterial hand washing products, 263
- antibiotics, 20, 96, 297
- anti-dandruff, 263, 264
- anti-dandruff agents, 273
- antifouling paints, 38, 113
- antimicrobial, 263
- antimicrobial action, 272
- antimicrobial agents, 263, 268
- antimicrobial efficacy, 268, 271
- antimicrobial efficacy of alcohol, 271
- antimicrobial enzymes, 41
- antimicrobial hand wash preparations, 264
- antimicrobials for plastics, 326
- antioxidants, 271
- antiperspirants, 265
- anti-plaque additives, 273
- antiseptic effect, 268
- antiseptics, 96
- antiseptic substances, 274
- AOX values, 323

- aroylethylene, 15
- ARTCA, 136
- artificial contamination, 278
- Aspergillus* sp., 352, 361, 363
- Aureobasidium* sp., 361, 363
- autoinducers, 100
- aviation fuel biocides, 195
- a_w value, 269
- Azaconazole, 26
- azole fungicides, 12
- azoles, 26

- babies, 267
- Bacillus, 352
- Bacillus subtilis*, 319
- bacteria, 122, 177
- bacterial degrade of wood, 422
- bacterial diversity, 255, 256
- bacterial morphologies, 254
- bacterial spores, 266, 280
- bactericides, 3, 30, 268
- bacteriostasis, 268
- Balanus improvisus*, 124
- basidiomycetes, 124
- batch mode, 102
- bath products, 264
- BCDMH, 128
- benzalkonium chloride, 272, 276
- benzethonium chloride, 272
- benzimidazole fungicides, 435
- benzisothiazolin-3-one (BIT), 18
- benzoic acid, 288
- benzothiophene-2-carboxamid-S,S-dioxides, 27
- benzoylureas, 35
- benzyl alcohol, 276
- beta-bromo-beta-nitrostyrene, 130
- Bethoxazin, 29, 436
- Bifenthrin, 35
- biguanides, 99
- bio-availability, 272
- biocidal blends, 130
- Biocidal Products Directive 98/8
EG (BPD), 65, 261
- biocidal program, 126
- biocide efficacy determination, 101, 103, 199
- biocide partitioning, 195
- biocide treatment, 101, 102
- biocides, 15, 96, 126, 192, 263
- biocides for plastics, 325
- biocorrosion, 94
- biodeterioration, 94
- biofilm assays, 101
- biofilm development, 253
- biofilm formation, 254
- biofilm monitoring, 125
- biofilm phenotypes, 94
- biofilm resistance, 97
- biofilms, 21, 41, 93, 94, 121, 122,
161, 253, 353, 364
- biofilm-specific phenotype, 100
- biofouling, 94
- biofouling control, 171
- biostable, 208
- biphenyl, 300
- biphenyl-2-ol, 301
- bis(tributyltin)oxide, 130
- bis(trichloromethyl) sulfone, 130
- BIT, 31
- blue-green algae, 228
- bluestain in service, 426
- BNPD, 129
- body odour, 263
- Boko test, 208
- booster biocides, 38, 41
- boron, 433
- breakpoint chlorination, 145
- broad spectrum efficacy, 275
- bromamines, 129
- bromine, 142
- bromine chloride, 128
- bromo-1-(bromomethyl)-1,3-
propanedicarbonitrile, 130
- Bromuconazole, 26
- Bronidox, 276
- Bronopol, 163, 272, 276
- Brown, 4
- brown rot, 124
- brown rot fungi, 424
- building-related illness, 361
- butylhydroxyanisol (BHA), 273
- butylhydroxytoluol (BHT), 273

- C-14 demethylase, 12
- calcium benzoate, 288
- calcium carbonate, 251
- calcium dipropionate, 291
- calcium hypochlorite, 128, 143, 147
- calcium sorbate, 289
- Candida albicans*, 352
- Candida* sp., 361
- Cannizzaro's reaction, 21
- carbendazim, 435
- carboxylates, 271
- carboxymethyl cellulose, 350
- catalase, 99
- catalase control, 395
- cationic biocides, 99
- cationic surfactants, 264, 271
- CCA, 432
- cell-cell communication, 100
- cellulases, 230
- cellulose derivatives, 264
- cetrimonium bromide, 272
- Cetyltrimethylammonium bromide, 276
- Chaetomium* sp., 361
- challenge tests, 278
- chelate formers, 12, 13
- chelating agents, 29, 33, 271
- chelation, 10
- chemical preservation, 204, 212

- chitin, 416
- chitin synthesis inhibitors (CSIs), 436
- Chitosan, 416
- chloramines, 129
- Chlorella, 364
- Chlorhexidin
- chlorinated cyanuric acid derivatives, 128
- chlorinated phenols, 322
- chlorine, 20, 98, 142
- chlorine-containing compounds, 104
- chlorine dioxide, 128, 152
- chlorine generators (chlorinators), 151
- chlorine releasing agents, 20
- Chlorococcum, 364
- chloromethylisothiazolinone/
methylisothiazolone (3:1), 274
- Chloronicotinyne, 37
- Chloropyriphos, 37
- chlorthalonil, 435
- chrome tannage, 317
- Chroococcus, 364
- Citrobacter* spp., 266
- Cl₂, 128
- Cladosporium* sp., 361, 363
- classes of biocides, 127
- cleaning and disinfection, 281
- cleaning efficacy, 103
- cleansing products, 264
- clostridia, 272
- clostridian spores, 266
- Clostridium perfringens*, 267
- Clostridium tetani*, 267
- coccoid bacteria, 264
- Coleoptera, 421
- COLIPA, 281
- colloids, 219
- colour changes, 230
- compounds with activated halogen atoms, 31
- concept, 269
- consumer health
- continuous mode, 102
- cooling towers, 123
- cooling water systems, 95, 121
- copper, 38
- copper-8-hydroxyquinoline, 13
- copper-azole, 433
- copper citrate, 433
- copper dimethyl dithiocarbamate, 433
- copper-n-cyclohexyldiazonium, 433
- copper quaternary ammonium compounds, 433
- copper/silver ionizers, 153
- copper sulfate, 142
- copper thiocyanate, 38
- Corbicula, 124
- Corbicula fluminea*, 125
- corrosion, 231
- cosmeceuticals, 263
- Cosmetic Guidelines (76/768/EEC), 273
- cosmetics with anti-microbial claims, 268
- Council of Europe, 281
- creams, 264
- creosote, 434
- critical micelle concentration, 221
- cross-contamination, 282
- crust, 317
- cultivation of biofilms, 101
- cuprous oxide, 38
- cyaniminodithiocarbonates, 27
- cyanobutanes, 243
- cyanuric acid, 147
- cycles of concentration, 121
- Cyfluthrin, 35
- Cypermethrin, 35
- Cyproconazole, 26
- cytochrom P-450, 12
- cytoplasm, 4, 10
- cytoplasmic membrane, 4, 10, 11, 14
- Dantobrom, 136
- DBNPA, 129, 130
- degrade of wood by beetles, 421
- degrade of wood by insects, 421
- degrade of wood by termites, 421
- dehydracetic acid, 299
- dehydroacetic acid, 275, 299
- Deltamethrin, 35
- deodorants, 263
- description of cosmetic products, 263
- Desulfovibrio* spp., 186
- DGH, 129
- dialdehydes, 14
- diatoms, 123
- dibromdicyanobutane, 274
- Dichlofluanid, 39
- dichlorobenzyl alcohol, 276
- Diclosan, 33
- Dictyna civica, 366
- didecyldimethylammonium-chloride, 321
- diethyldicarbonate, 298
- diffusion coefficient, 10
- Diflubenzuron, 35
- dihydrouracil, 15
- dimethyldicarbonate (DMDC), 298
- dimethylpyrocarbonat, 298
- diphenyl, 300
- disinfectants, 96, 141, 268, 305
- disinfection, 204, 271, 282
- dismutation, 21
- dispenser system, 278
- dispersants, 252, 256
- disproportionation, 99
- dissolved oxygen (DO), 131
- distribution coefficient, 6
- Dithiocarbamates, 129
- DMDM-hydantoine, 276
- dodecyldimethyl ammonium chloride, 154
- dodecylguanidine hydrochloride, 130
- Dowicil, 200, 276
- DPD (N,N'-diethyl phenylenediamine)
method, 146

- Dreissena polymorpha*, 125
 drinking water distribution systems, 95
 droplet size, 264
 drums and containers, 235
 DTEA, 129
 durability, 420
 D-value, 280
- easy-care coolants, 208, 209
 ecdysone antagonist, 37
 EDTA, 271, 273
 efficacy of preservatives, 267
 efficacy spectrum, 274
 Ehrlich, Paul, 9
 EIFS, 348, 365
 electrolytes, 264
 electronegative group, 16
 electronic cell counting, 256, 258
 electrophilically active microbicides, 6, 13, 14, 19, 21
 electrophilically active substances, 17
 electrophilicity, 14, 17
 electrophilic substances, 31
 emollients, 272
 emulsion polymerisation, 221
 emulsions, 264
Enterobacter spp., 266
Enterobacter coli, 265
 enzymatic slime control, 393
 enzyme inhibitors, 265
 enzyme production, 230
 enzymes, 99, 266
 EPA, 136, 437
Epicoccum, 363
 ergosterol, 12, 26
 ergosterol biosynthesis, 12
Escherichia coli, 319, 352
 esters of p-hydroxybenzoic acid, 294
 Ethancarboxylic acid, 291
 ether sulphates, 271
 Ethyl 4-hydroxybenzoate, 294
 ethylene glycol hemiformal, 259, 260
 ethylene oxides, 20
 ethyl-urethan, 298
 EU Directive, 263
 European hazard class system, 421
 evaluation criteria, 278
 evaluation of polymer dispersion biocides, 246
 evaluation of preservatives in the
 marine environment, 429
 exopolysaccharide glycoalkyl polymers, 21
 export of antimicrobials, 61
 exposure assessment, 84
 extracellular polymeric substances (EPS),
 21, 93, 98, 131
 extracellular polysaccharide (EPS), 380
 eye products, 267
- facilitated transport, 10, 13
 facultative bacteria, 380
 facultatively anaerobic microorganisms, 256
- Failure Mode and Effects Analysis (FMEA), 282
 Farnesol, 273
 fault prevention analyses, 282
 FDA, 136
 fenoxycarb, 36
 field evaluation methodologies, 428
 FIFRA, 136
 filamentous bacteria, 380
 filamentous mould, 123, 124
 Fipronil, 37
 floating biofilms, 93
 Flufenoxuron, 35
 fluorfolpet, 17
 food industry, 95
 formaldehyde, 20, 164, 241, 256, 260, 261, 276
 formaldehyde dehydrogenase, 21
 formaldehyde dismutase, 21
 formaldehyde donors, 241
 formaldehyde-releasing compounds, 260, 276
 formate, 256
 formic acid, 275
 foul-release coatings, 38
 FQPA, 136
 Frazer, 4
 fuel distribution system, 177
 fuel-soluble biocides, 194
 functional product damage, 266
 fungal degrade of wood, 423
 fungal toxins, 266
 fungi, 124, 177
 fungicides, 3, 25, 268
 fungistasis, 268
Fusarium sp., 352, 361, 363
- gas formation, 229
 general stress response, 99
 Geotrichum, 353
 Germall, 272
 Gleocapsa, 364
Gluconoacetobacter, 228
Gluconoacetobacter liquefaciens, 243
 glucose oxidase, 41
 glutaraldehyde, 20, 129, 130, 164, 260
 glycerol, 272
 glycerol monolaurate, 273
 glycol, 272
 Glydant, 272
 Gram-negative bacteria, 4, 20, 256, 267
 Gram-negative cells, 9
 Gram-positive bacteria, 4, 11, 20, 21, 256, 267
 Gram-positive *Micrococcus*, 256
 Gram-positive mycobacteria, 9, 11
 Guidelines for good manufacturing
 practice (CGMP), 265, 267, 281
- hair conditioners, 264
 haloalkylsulfenylchlorides, 17
 halogen biocides, 128
 halogenated phenols, 559
 hardwoods, 420

- Hazard Analysis Critical Control Points (HACCP), 282
- heath risk, 267
- health safety and the environment, 437
- heat transfer, 123
- heat transfer resistance (HTR), 131
- heavy metal based biocides, 240
- hemi-formals, 214
- heterocyclic N,S compounds, 322
- Hexaconazole, 26
- Hexaflumuron, 35
- hexahydrotriazines, 214
- Hexetidine, 273
- high performance liquid chromatography (HPLC), 223
- high-throughput screening, 42
- homogenisation of biofilm cells, 101, 103
- Hormoconis resinae*, 177
- housekeeping, 251, 256
- humectants, 264, 272
- hydrocarbon-utilizing microorganisms, 179
- hydrogen peroxide, 98, 128, 142, 237, 271
- hydroviscous coatings, 41
- hygiene conditions, 283
- hygiene recommendations, 283
- hygiene technology, 281
- hypochlorite ion (OCl^-), 144
- hypochlorites, 20
- hypochlorous acid (HOCl), 144

- Imidacloprid, 37, 436
- Imidazoles, 26
- import of antimicrobials, 61
- improving plant hygiene, 237
- inactivation, 280
- incubation, 280
- infections, 265
- ingredients, 278
- initiators, 219, 222
- inoculation volumes, 280
- inoculum, 279
- inorganic bactericides, 34
- insect growth regulators (IGR), 436
- insecticides, 34
- International Biodeterioration Research Group (IBRG), 227
- International Maritime Organisation (IMO), 373
- intrinsic resistance, 20, 97
- in-use tests, 278, 281
- iodine, 151
- iron and manganese oxidizing bacteria, 161
- irradiation sterilisation, 272
- irritation, 274
- Isoptera, 421
- isothiazolin (MIT/CIT/BIT), 259
- isothiazolinone derivatives, 17
- isothiazolinone ring, 18
- isothiazolinones, 276
- isothiazolones, 129, 434

- juvenile hormone, 36
- juvenile skin problems, 264

- kaolin, 251
- Kathon CG, 272
- killing efficacy, 103
- Klebsiella*, 267
- Klebsiella pneumoniae*, 98
- Klebsormidium, 364

- labeling of antimicrobials, 54
- lactic acid bacteria, 296
- lactoperoxidase, 41
- lanosterol, 12
- Lantibiotics, 298
- latexes, 219
- leather, 317
- leaving group, 16
- Leeuwenhoek, 4
- Legionella*, 96, 123
- Legionella pneumophila*, 123
- levels of microorganisms, 267
- Limnoria*, 426
- Lister, 4
- lithium hypochlorite, 143, 147
- lotions, 264

- macrofouling, 124
- macro-invertebrates, 121
- Magnusson and Kligman, 246
- mains water, 278
- malodours, 230
- manufacturing hygiene, 267
- manufacturing process, 263
- marine borer damage to wood, 425
- Marine Environmental Protection Committee (MEPC), 373
- marine fouling, 124
- medical devices, 95
- membrane active agents, 21
- membrane active compounds, 30
- membrane active microbicides, 6, 10, 14
- membrane active substances, 20
- mercury, 240
- metal-based wood preservative system, 432
- metallic compounds, 415
- metalworking fluids, 5
- Metconazole, 26
- methane, 256
- methane bacteria, 3
- methanol, 256
- Methanotrophs, 256
- methods of assessment for wood preservatives, 426
- Methyl 4-hydroxybenzoate, 294
- Methylacetic acid, 291
- methylamine, 256
- methylene bistiocyanate (MBT), 130, 261, 435
- Methylotrophs, 256
- Methylparaben, 294
- methyl-urethan, 298

- mica, 251
- micelle, 221
- microbe inhibitors, 265
- microbial activity (ATP) test, 401
- microbial aggregates, 93
- microbial contamination, 177, 263
- microbial degradation of plastics, 325
- microbial resistance to microbicides, 20
- microbial spoilage, 269
- microbial toxins, 266
- microbial volatile organic compounds (MVOC), 361
- microbially influenced corrosion (MIC), 94, 121
- microbicidal, 3, 206
- microbicides, 3, 268
- microbiological acceptance criteria, 268
- microbiological degradation, 177
- microbiological safety, 265
- microbiology of polymer dispersions, 224
- microbistasis, 268
- microbistatic, 3, 206
- microbistats, 3
- Micrococcus*, 319, 352
- microfouling, 122
- microtextured coatings, 41
- mineral dispersions, 251, 256
- mineral raw materials, 272
- mineralization, 3
- minimum inhibitory concentration (MIC), 274
- minimum microbicidal concentration (MMC), 274
- mixing vessels and storage tanks, 234
- monitor, 115
- monoaldehydes, 14
- monochloro-o-phenylphenol, 559
- monoglyceride derivatives, 271
- monomers, 219
- monothiomalonic acid monoamide derivatives, 18
- moulds, 179, 275, 320
- mould and staining fungi, 423
- Mucor* sp., 361
- multicide inhibitors, 27
- mutants, 20
- mutations, 20
- Myavert[®] C, 41
- mycotoxins, 272
- Mytilus edulis*, 124

- N-(3,4-dichlorophenyl)-N'-acryl-urethane, 15
- Na-hydroxymethylglycinate, 274
- NaOCl, 128
- NaOCl + NaBr, 128
- natural antifouling compounds, 114
- n-butyl-BIT (BBIT), 32
- N-Cyclohexyl-benzothiofen-2-carboxamid-S,S-dioxide, 27
- neutralization, 145
- N-formals, 214
- N-haloalkylthio compounds, 17
- N-hydroxymethyl-N-methyldithiocarbamate, 321
- nisin, 295
- nitrate, 293
- nitrite, 293
- nitrosating, 215
- non-anionic surfactants, 222
- non-ionic surfactants, 272
- non-oxidizing biocides, 129, 384
- norms, 268
- Nostoc, 364
- nucleophiles, 16
- nucleophilic cell components, 15, 16
- nucleophilic components, 6, 14, 21
- nutrient limitation, 99

- official regulations, 267
- O-formals, 214
- oilfield application for biocides, 157
- oligo-dynamic action, 415
- o-phenylphenol, 260, 301
- oral care products, 265
- oral cavity, 267
- oral hygiene, 263
- organic wood preservative chemicals, 433
- organometallic compounds, 4, 19
- organostannic compounds, 415
- organotin compounds, 38
- ORP (oxidation-reduction potential), 146
- ortho-phenylphenol (OPP), 322, 323
- Oscillatoria, 364
- OSHA, 357
- OTC drugs, 268
- other uses, 273
- OTO (orthotolidine) method, 146
- outer membrane, 4, 20
- overdose, 207
- O/W emulsion, 264
- oxidizers, 127
- oxidizing agents, 20
- oxidizing biocides, 98, 127, 128
- oxine, 13
- oxine copper, 13
- ozone, 107, 142, 151, 271

- packaging, 265, 269
- Paecilomyces*, 363
- parabens, 294
- para-Chloro-m-cresol, 30
- paraformaldehyde, 214
- parahydroxybenzoic acid esters (Parabens), 277
- parallel synthesis, 42
- partition coefficient, 6, 274
- passive transport, 10
- Pasteur, 4
- pathogenic bacteria, 204
- pathogenic organisms, 267
- p-chloro-m-cresol (PCMC), 322
- penetration of preservatives, 420
- Penicillium* sp., 353, 361, 363
- pentachlorophenol (PCP), 5, 322, 435
- peracetic acid, 108, 128, 237
- perfume oils, 273

- periplasm, 14
- Permethrin, 35
- peroxides, 20
- peroxygens, 107
- persister cells, 100
- pharmacopoeias, 278
- PHB esters, 274
- pH changes, 229
- pH potential, 270
- pH values, 270, 274
- phenol, 260
- phenol derivatives, 4, 20, 260, 277
- phenoxyethanol, 273
- phenylethylalcohol, 272
- phenylpyrazole, 37
- phenylsulphamide fungicides, 435
- Phoma, 363
- Phormidium, 364
- p*-hydroxy-benzoic acid, 20
- physical-chemical conditions, 269
- physical treatment, 204
- Pigment-Volume-Concentration (PVC), 348
- pimaricin, 296
- pipelines and hoses, 234
- piroctone olamine, 273
- pK_a values, 10
- planktonic assays, 101
- planktonic bacteria, 94, 382
- planktonic cells, 21
- planktonic organisms, 131
- plant design, 234
- plant extracts, 264
- plant hygiene, 237
- plaque, 265
- plaque formation, 265
- plasticizer, 5
- Pleurococcus, 364
- polyacrylic acid (PAA), 252, 256
- polyhexamethylene biguanide, 142, 143
- Polymer Dispersion Group, 227
- polymer dispersions, 219
- Polymerase Chain Reaction (PCR), 254, 255
- polymeric materials, 325
- poly(oxy)ethylene(dimethylimino)ethylene(dimethyl-amino)ethylene dichloride, 153
- poly quat, 153
- Polyurethanes, 327
- polyvinyl alcohol, 219, 222, 233
- potassium benzoate, 288
- potassium (E,E)-sorbate, 289
- potassium monopersulfate, 145
- potassium nitrate, 293
- potential risk, 284
- potential vinyl compounds, 16
- precipitated calcium carbonate, 251
- Predicted Environmental Concentration (PEC), 79
- preservation, 204, 263
- preservative challenge testing, 266, 267
- preservative protection, 266
- preservatives, 263, 267, 273
- preservative systems, 263, 267
- prevention, 115
- Preventol[®] TP OC 3082, 27
- prions, 266
- product contamination, 266
- product preservation, 263, 265, 270
- product spoilage, 263
- product surface, 275
- production hygiene, 263, 267, 281
- production water, 270
- programmed cell death, 100
- Propiconazole, 26
- Propionibacterium acnes*, 264
- Propionic acid, 290
- Propyl 4-hydroxybenzoate, 294
- Propylparaben, 294
- protection, 269
- protection of textiles, 411
- proteins, 264
- proteolytic enzymes, 296
- Proteus vulgaris*, 319, 352
- prototropic tautomerisation, 19
- pseudomonads, 256
- Pseudomonas aeruginosa*, 7, 20, 97, 267, 319
- Pseudomonas putida*, 20, 278
- Pseudomonas* spp., 177, 266, 352
- public health, 305
- pyrethroids, 34, 436
- pyrithione, 12
- pyrithionedisulfide, 12, 13
- pyrithionedisulfinate, 12, 13
- pyrithione sulfonic acid, 12
- quaternary amines, 142
- quaternary ammonium compounds (QACs), 99, 130, 166, 272, 276
- quaternary ammonium salts, 20
- quats, 129
- quorum sensing, 42, 100
- RAL UZ, 102, 357
- raw material contamination, 265
- raw material quality, 278
- raw materials, 265
- ray tissues, 420
- rDNA Internal Spacer Analysis (RISA), 254, 255, 256
- reaction-diffusion interaction, 98
- real-time monitoring, 256, 258
- recreational water, 141
- redox, 223
- redox potential, 270, 387
- re-fill systems, 278
- registration of antimicrobials, 51
- regulation of antimicrobials, 51
- regulations in the EU, 268
- regulatory compliance, 51
- regulatory negotiation, 58
- removal of biofilm cells, 101, 103

- reregistration of antimicrobials, 47
- residual monomer, 226
- resistance, 97, 207, 266
- resonance-stabilized system, 15
- Rhizopus, 363
- Rhodotorula rubra*, 352
- Rhodotorula* sp., 361
- risk assessment, 79, 80, 86
- Risk Characterisation Ratios (RCR), 79
- risk to health, 263, 267
- road and rail tankers, 235
- Saccharomyces cerevisiae*, 352
- salicylic acid, 273
- sanitizers, 141, 305
- Scenedesmus, 364
- screening, 43
- Scytonema, 364
- Sea-Nine, 39
- selection, 20, 208
- self-polishing coatings, 40
- self-preservative properties, 265
- semi-volatile organic compound (SVOC), 359
- Serratia*, 267
- Serratia marcescens*, 352
- sessile bacteria, 382
- sessile microorganisms, 125
- shampoos, 264
- shock, 141
- sick-building syndrome, 361
- silver, 142, 151, 154
- silver compounds, 108
- skin compatibility, 274
- skin-sensitisation, 261, 274
- slime, 93, 121
- slime-forming bacteria, 123, 380
- slow growth rate, 99
- sodium benzoate, 273, 288
- sodium bromide, 149
- sodium dichloroisocyanurate (dichlor), 148
- sodium dichloro-s-triazinetriene, 143
- sodium dimethyldithiocarbamate, 321
- sodium hypochlorite, 143, 147
- sodium metabisulfite, 292
- sodium nitrite, 293
- sodium propionate, 291
- sodium sulphite, 292
- soft rot, 124
- softwoods, 419
- soil rot fungi, 424
- sorbic acid, 275, 289
- Sphaerotilus natans*, 131
- spoilage, 266
- spore-forming bacteria, 123
- spores, 20, 272, 353
- sporicidal effects, 20
- sporicidal microbicides, 20
- sporistatic, 20
- sporogenic species, 20
- sporulation, 20
- stabilized chlorine, 147
- Stachybotris* sp., 361
- staining of flexible PVC, 329
- Stemphylium, 363
- Staphylococcus*, 256
- Staphylococcus aureus*, 20, 264, 319
- Staphylococcus epidermis*, 264
- Stitchococcus, 364
- Streptovorticillium reticulum*, 329
- structural degradation of plastics, 332
- Subtilin, 298
- succession, 353
- sugar surfactants, 264
- sulfate reducing bacteria, 161, 177
- sulfites, 292
- sulfur aerobic bacteria, 123
- sulfluramid, 36
- sulfur dioxide, 292
- sulfurous anhydride, 292
- sulphate-reducing bacteria, 3, 123
- sulphates, 271
- sulphonates, 271
- sun protection, 264
- superchlorination, 145
- surface-active microbicides, 11
- surface-bound biocides, 113
- surface effect, 275
- surface-generated biocides, 114
- surfactants, 220, 271
- surfactants and/or colloids, 219
- syneresis, 229
- synergism index, 130
- synergistic combinations, 130
- system cleaning, 204
- talc, 251
- tanning, 317
- Tebuconazole, 26
- Tebufenocide, 37
- technical water systems, 95
- temperature tolerance, 275
- test cultures, 279
- test methods, 146
- Tetraconazole, 26
- tetracyclines, 298
- tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione, 321
- thiazole/benzothiazole fungicides, 435
- Thiazolylpyrazolones, 29
- thickening agents, 264
- thiabendazole, 299
- thiamethoxam, 436
- thioacrychlorides, 19
- thiohydroxamic acids, 33
- thiocarbamoylpyrazolones, 29
- thioclopid, 436
- thiocyanates, 129
- THPS, 129, 166
- tinting pastes, 350
- Tolylfluanid, 38

- tolylfluamide, 17
- toothpastes, 265
- toxicology of the preservatives, 208
- toxins, 267
- toxophoric, 9, 14
- toxophoric groups, 9
- toxophoric structural element, 15, 19
- transport limitation, 98
- trentepholia, 364
- TRGS, 611, 215, 216
- triazole fungicides, 434
- triazoles, 26
- Tribonema, 364
- tributyltin(TBT), 38
- trichlor, 148
- trichloroisocyanuric acid, 148
- trichloro-s-triazinetriene, 143, 148
- Triclosan, 33, 273, 277
- Trichoderma* sp., 361
- Triflumuron, 35
- Trilocarban, 274
- tri-n-butyl tin oxide, 433
- trioxan, 214
- tris(aroylethyl)amine, 15
- TTC, 273
- Tylosin, 298

- Ulocladium, 363
- ultrapure water, 95
- ultraviolet light (UV), 142, 152
- ultraviolet sterilisation, 236
- UV irradiation, 271
- underdose, 207
- unsaturated aldehyde, 15

- unstabilized chlorines, 146

- validation, 263, 279
- validation of effective preservation, 277
- vegetable tanning, 317
- viable but non-culturable (VBNC), 103
- vinyl group, 15
- viruses, 266
- viscosity changes, 229
- visible surface growth, 230
- vital counting, 256
- vital staining, 258

- water, 270
- water cooling systems, 126
- water-free powders, 266
- water-free products, 269
- water-soluble biocides, 194
- water solubility, 274
- wet blue, 317
- wet white, 317
- white rot, 124
- white rot fungi, 425
- W/O emulsion, 264
- wood decay fungi, 424
- wood preservative design, 430
- wood preservative formulations, 430
- wood protection, 419
- wood structure and chemistry, 419

- yeasts, 123, 179, 320

- zebra mussel, 124
- zinc pyrithion, 273