REGULATION (EC) NO 1272/2008 (CLP REGULATION),

ANNEX VI, PART 2

Proposal for Harmonised Classification and Labelling for a biocidal active substance

CLH REPORT

bronopol; 2-bromo-2-nitropropane-1,3-diol

EC Number: 200-143-0

CAS Number: 52-51-7

Index Number: 603-085-00-8

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SUMMARY

1 PRESENTATION OF THE ACTIVE SUBSTANCE

1.1 IDENTITY OF THE ACTIVE SUBSTANCE

Table 1-1: Main constituent

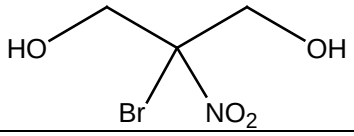
Main constituent	
ISO name	-
IUPAC or EC name	bronopol; 2-bromo-2-nitropropane-1,3-diol
EC number	200-143-0
CAS number	52-51-7
Index number in Annex VI of CLP	603-085-00-8
Minimum purity / content	≥98.90% w/w
Structural formula	C3H6BrNO4 

Table 1-2: Relevant impurities and additives
No impurity or additive that contributes to the classification.

Impurities: The identity and content of the impurities is an industrial and commercial secret. Therefore, this information should be treated confidential.

Additives: The technical grade active ingredient does not contain additives.

1.2 INTENDED USES AND EFFECTIVENESS

Table 1-3: Use of the active substance

Product type	2
Intended use pattern(s)	Disinfection of chemical toilets Bronopol is used for the disinfection of chemical toilets where faeces are collected in tanks and sanitary additives containing biocides are added for disinfection and reduction of odour. Chemical toilets may be installed on transport vehicles (e.g. long distant busses, camping vans), at temporary sites (e.g. camping sites), or at other places without any possibility of a direct connection to the sewer system. Application: The sanitary additives together with a certain amount of water (depending on the actual product and the size of the respective tank) are filled into the sewage tank of the chemical toilet as so-called pre-charge.
Users	Disinfectant produced by applicants: Industrial and professional users Sanitary additive produced by downstream-user: Professional and non-professional users

Table 1-4: Effectiveness of the active substance

Function	Bactericide, used to control the growth of bacteria
Organisms to be controlled	Malodour producing bacteria commonly present in chemical toilets This covers gram-negative bacteria like *Escherichia coli, Enterobacter aerogenes* and *Pseudomonas aeruginosa* as well as gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus*.
Limitation of efficacy including resistance	Bronopol is stable in acidic conditions, but chemically less stable in alkaline systems. Due to the complex mode of action of Bronopol, no resistance development is to be expected.
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Bronopol catalytically oxidises thiol-groups to disulphide bonds with rapid consumption of oxygen. Bronopol is not destroyed during the oxidation of thiol-groups. If the thiol-groups are too far apart or lie in close proximity to electronegative polar groups, oxidation will not occur or be hindered. In the absence of air (oxygen), Bronopol seems to act as an oxidizing agent. Reduction of growth rate following the induced bacteriostasis probably reflects irreversible damage to the cell, possibly through the generation of oxygen radicals. The results suggest a dual action of Bronopol, with catalytic oxidation of accessible thiols being responsible for the growth inhibition and generation of free radicals causing cell death.

Table 1-5: Use of the active substance

Product type	6 (sub-PT 6.1)
Intended use pattern(s)	In-can preservation of washing and cleaning fluids and human hygienic products Bronopol can be used as an in-can preservative in a variety of PT 6 products (e.g. sub-PTs 6.1 and 6.2). For the active substance approval, the Intended Use described by sub-PT 6.1 has been selected for its evaluation. This sub-category covers in-can preservatives used to preserve detergents and cleaning products. Preservation of such products is carried out to prevent deterioration during storage in containers. Application: During the formulation of the preserved product (e.g. surface cleaner), the biocidal product is homogenously incorporated into the end-product. In some cases, the biocidal product may be diluted prior to its incorporation.
Users	Industrial and professional users

Table 1-6: Effectiveness of the active substance

Function	Bactericide, to control the growth of potentially pathogenic microorganisms responsible for spoilage during the shelf life of the product
Organisms to	Spoilage causing bacteria in detergents and human hygienic products

be controlled	This covers gram-negative bacteria like *Escherichia coli, Enterobacter aerogenes* and *Pseudomonas aeruginosa* as well as gram-positive bacteria like *Enterococcus hirae* and *Staphylococcus aureus*. Activity against moulds (*Aspergillus niger*) and yeasts (*Candida albans*) could also be shown, but to a lesser extent than the anti-bacterial activity. Within this AR efficacy against fungi has not been sufficiently supported. At the product authorization stage efficacy against yeasts and moulds should be verified in applications where it is claimed.
Limitation of efficacy including resistance	Bronopol is stable in acidic conditions, but chemically less stable in alkaline systems. However, in-use experience has shown that Bronopol is an effective preservative in alkaline systems. At pH above about 8.5-9.0, Bronopol will not be long lasting as an in-can preservatives due to lack of chemical stability. Resistance are not expected due to the complex mode of action of the active substance.
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Bronopol catalytically oxidises thiol-groups to disulphide bonds with rapid consumption of oxygen. Bronopol is not destroyed during the oxidation of thiol-groups. If the thiol-groups are too far apart or lie in close proximity to electronegative polar groups, oxidation will not occur or be hindered. In the absence of air (oxygen), Bronopol seems to act as an oxidizing agent. Reduction of growth rate following the induced bacteriostasis probably reflects irreversible damage to the cell, possibly through the generation of oxygen radicals. The results suggest a dual action of Bronopol, with catalytic oxidation of accessible thiols being responsible for the growth inhibition and generation of free radicals causing cell death.

Table 1-7: Use of the active substance

Product type	11
Intended use pattern(s)	Preservatives for liquid-cooling and processing systems Bronopol is used as a cooling water preservative (e.g. in open and closed recirculating cooling systems). Preservative treatment with continuous dosing as well as curative treatment with shock dosing is intended. It is not known that the substance is also used for once-through cooling systems. Within this assessment report, preventive use is supported. Application: The biocidal product may be applied directly or, alternatively, as pre-mix into the water matrix to be preserved. A homogenous incorporation of the active substance into the system being treated is to be ensured.
Users	Industrial and professional users

Table 1-8: Effectiveness of the active substance

Function	Bactericide, to control proliferation of potentially pathogenic microorganisms in cooling and processing systems
Organisms to be controlled	Typical target organisms are bacteria, algae and fungi. The main target organism from a public health standpoint is *Legionella pneumophila.* More common organisms are gram-negative bacteria such as *Enterobacter cloacea, Aeromonas hydrophila* and *Pseudomonas aeruginosa* as well as gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus hirae*. Fungi: *Aspergillus niger, Chaetomium globosum, Cladosporium herbarum, Stachybotrys atra* and species of genus *Penicillium* and *Trichophyton.* Within this AR only innate efficacy of the active substance against fungi has been demonstrated. Algal species: *Scenedesmus obliquus*, *Chlorella emersonii var. Globosa* and *Euglena gracilis.* Within this AR only innate efficacy of the active substance against *Scenedesmus obliquus* and *Chlorella emersonii* has been demonstrated.
Limitation of efficacy including resistance	Bronopol is stable in acidic conditions, but chemically less stable in alkaline systems. However, in-use experience has shown that Bronopol is an effective preservative in alkaline systems. At pH above about 8.5-9.0, Bronopol will not be long lasting as an in-can preservatives due to lack of chemical stability. Resistance are not expected due to the complex mode of action of the active substance.
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Bronopol catalytically oxidises thiol-groups to disulphide bonds with rapid consumption of oxygen. Bronopol is not destroyed during the

	oxidation of thiol-groups. If the thiol-groups are too far apart or lie in close proximity to electronegative polar groups, oxidation will not occur or be hindered. In the absence of air (oxygen), Bronopol seems to act as an oxidizing agent. Reduction of growth rate following the induced bacteriostasis probably reflects irreversible damage to the cell, possibly through the generation of oxygen radicals. The results suggest a dual action of Bronopol, with catalytic oxidation of accessible thiols being responsible for the growth inhibition and generation of free radicals causing cell death.

Table 1-9: Use of the active substance

Product type	12
Intended use pattern(s)	Slimicides Bronopol is used for the prevention and the control of slime growth on materials, equipment and structures, used in industrial processes (e.g. in paper mills). Preservative treatment with continuous dosing as well as curative treatment with shock dosing is intended. Within this assessment report, preventive use is supported. Application: The biocidal product may be applied directly or, alternatively, as pre-mix into the water circuit to be preserved – ideally to the primary white water circuit. A homogenous incorporation of the active substance into the system being treated is to be ensured.
Users	Industrial and professional users

Table 1-10: Effectiveness of the active substance

Function	Slimicide, to control microbially induced damage to plant / equipment, pipework during industrial and cooling processes
Organisms to be controlled	Typical target organisms are gram-negative bacteria such as *Enterobacter aerogenes, Aeromonas hydrophila* and *Pseudomonas aeruginosa* as well as gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus hirae.* Fungi: *Aspergillus niger, Chaetomium globosum, Cladosporium herbarum, Stachybotrys atra* and species of genus *Penicillium* and *Trichophyton.* Within this AR only innate efficacy of the active substance against fungi has been demonstrated. Algal species: *Scenedesmus obliquus*, *Chlorella emersonii var. Globosa* and *Euglena gracilis.* Within this AR only innate efficacy of the active substance against *Scenedesmus obliquus* and *Chlorella emersonii* has been demonstrated.
Limitation of efficacy including resistance	Bronopol is stable in acidic conditions, but chemically less stable in alkaline systems. Due to the complex mode of action of Bronopol, no resistance development is to be expected.
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Bronopol catalytically oxidises thiol-groups to disulphide bonds with rapid consumption of oxygen. Bronopol is not destroyed during the oxidation of thiol-groups. If the thiol-groups are too far apart or lie in close proximity to electronegative polar groups, oxidation will not occur or be hindered. In the absence of air (oxygen), Bronopol seems to act as an oxidizing agent. Reduction of growth rate following the induced bacteriostasis probably reflects irreversible damage to the cell, possibly through the generation of oxygen radicals. The results suggest a dual action of Bronopol, with catalytic oxidation of accessible thiols being responsible for the growth inhibition and generation of free radicals causing cell death.

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO THE CLP CRITERIA

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING FOR THE ACTIVE

Table 2-1: Proposed harmonised classification and labelling of the substance

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	603-085-00-8	bronopol (INN); 2-bromo-2-nitropropane-1,3-diol	200-143-0	52-51-7	Acute Tox. 4* Acute Tox. 4* STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1	H312 H302 H335 H315 H318 H400	GHS05 GHS07 GHS09 Dgr	H312 H302 H335 H315 H318 H400		M=10	
Dossier submitters proposal	603-085-00-8	bronopol; 2-bromo-2-nitropropane-1,3-diol	200-143-0	52-51-7	**Retain** STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 **Add** Acute Tox. 3 Aquatic Chronic 1 **Modify** Acute Tox. 4 Acute Tox. 3	**Retain** H335 H315 H318 H400 **Add** H331 H410 **Modify** H312 H301	**Retain** GHS05 GHS07 GHS09 Dgr **Modify** GHS06	**Retain** H335 H315 H318 **Add** H331 **Modify** H312 H301 H400 to H410	**Add** EUH044	**Add** inhalation: ATE = 0.588 mg/L (dust/mist) dermal: ATE = 1600 mg/kg bw oral: ATE = 193 mg/kg bw M=10 **Modify** M=10 to M=100	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	603-085-00-8	bronopol; 2-bromo-2-nitropropane-1,3-diol	200-143-0	52-51-7	Acute Tox. 3 Acute Tox. 4 Acute Tox. 3 STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H312 H301 H335 H315 H318 H400 H410	GHS05 GHS06 GHS09 Dgr	H331 H312 H301 H335 H315 H318 H410	EUH044	inhalation: ATE = 0.588 mg/L (dust/mist) dermal: ATE = 1600 mg/kg bw oral: ATE = 193 mg/kg bw M=100 M=10	

Spain	2-bromo-2-nitropropane-1,3-diol (Bronopol)

* Indication that at least this minimum classification must be applied, but classification in a more severe hazard category may be applied in the event that further information is available which shows that the hazard(s) meet the criteria for classification in the more severe category (see Annex VI, Section 1.2.1 of the CLP Regulation).

Table 2-2: Reason for not proposing harmonised classification and labelling and the status under CLH consultation

Hazard class	Reason for not proposing classification and labelling	Within the scope of consultation *(please select YES or NO from the drop down list)* (yes/no)
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable (e.g. physical state or chemical structure)	No
Oxidising gases	Hazard class not applicable (e.g. physical state or chemical structure)	No
Gases under pressure	Hazard class not applicable (e.g. physical state or chemical structure)	No
Flammable liquids	Hazard class not applicable (e.g. physical state or chemical structure)	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances and mixtures	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable (e.g. physical state or chemical structure)	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances and mixtures	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable (e.g. physical state or chemical structure)	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data conclusive but not sufficient for classification	Yes
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Desensitised explosives	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Harmonised classification proposed	Yes
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Harmonised classification proposed	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Harmonised classification proposed	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes

Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not applicable (e.g. physical state or chemical structure)	No

2.1.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has an entry in Annex VI to CLP as described in Table 2-1 of the CLH report and was introduced there with the 1st ATP to CLP.

2.2 PROPOSED CLASSIFICATION AND LABELLING AND PACKAGING FOR THE REPRESENTATIVE PRODUCT(S)

Not applicable for the CLH report.

2.3 DATA SOURCES

Active substance C&L: harmonised classification and labelling according to Regulation (EC) No 1272/2008 and available studies conducted with Bronopol.

Product C&L: based on the active substance C&L (representative product(s) contain 100% active substance).

Product Packaging: Specification Sheets of the companies.

Relevant information in REACH registration dossier were considered in preparation of the report as stipulated in Part 2 of Annex VI to the CLP Regulation.

3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

Not applicable for the CLH report.

4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

Not applicable for the CLH report.

5. ASSESSMENT OF EXCLUSION CRITERIA, SUBSTITUTION CRITERIA AND POP

Not applicable for the CLH report.

A Assessment of intrinsic properties and effects of the active substance

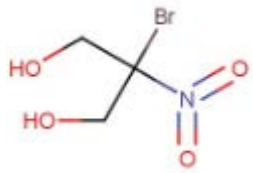
A.1 General substance information

A.1.1 Identity of the Substance

Table A-1: Summary table on substance identity

Summary table on substance identity	
Common name (ISO name, synonyms)	Bronopol BNPD
Chemical name (EC name, CA name, IUPAC name)	2-bromo-2-nitropropane-1,3-diol
EC number	200-143-0
CAS number	52-51-7
other CAS numbers (e.g. deleted, related, preferred, alternate)	--
Molecular formula	$C_3H_6BrNO_4$
Molecular weight or molecular weight range	199.9 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The substance is not chiral/isomeric.
Description of the manufacturing process and identity of the source (for UVCB substances only)	n.a.; substance is not an UVCB
Degree of purity (%)*	≥98.90% w/w

Table A-2: Structural formula

Structural formula


A.1.2 Composition of the substance (reference specifications)

Table A-3: Main constituent

Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
2-bromo-2-nitropropane-1,3-diol	≥98.90% (w/w)		Acute Tox. 4* (H302) Acute Tox. 4* (H312) STOT SE 3 (H335) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) Aquatic Acute 1 (H400)	Acute Tox. 3 (H301) Acute Tox. 3 (H331) Acute Tox. 4 (H312) STOT SE 3 (H335) Skin Irrit. 2 (H315) Eye Dam. 1 (H318) Aquatic Acute 1 (H400) Aquatic Chronic 2 (H411)	

* In line with the Regulation (EC) No 1272/2008, certain harmonized classifications marked with an asterisk (in Part 3 of Annex VI to CLP) are minimum classifications and, based on available data, a more severe classification as well as the corresponding hazard statement may need to be assigned.

Table A-4: Impurities

Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
n/a	n/a	n/a	n/a	n/a	n/a

Table A-5: Additives

Constituent (chemical name)	Typical concentration (%(w/w))	Concentration range (%(w/w))	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	Remarks / Discussion
n/a	n/a	n/a	n/a	n/a	n/a

A.1.3 Physical and chemical properties of the active substance

Table A-6: Physical and chemical properties of the active substance

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Aggregate state at 20 °C and 101.3 kPA	pellets	Visual inspection, GLP, purity 99.7%		[redacted] 2000 (A3.01.1_02)
	crystalline	Visual determination, GLP, purity: 98.7%		[redacted] 2001 (A3_1_1-01)
Physical state (appearance) at 20 °C and 101.3 kPA	solid	Visual inspection, GLP, purity 99.7%		[redacted] 2000 (A3.01.1_02)
	solid	Visual determination, GLP, purity: 98.7%		[redacted] 2001 (A3_1_1-01)
Colour at 20 °C and 101.3 kPA	At room temperature, the test substance consists of white pellets. It is obviously homogeneous.	Visual inspection, GLP, purity 99.7%		[redacted] 2000 (A3.01.1_02)
	White to yellowish crystalline solid	Visual determination, GLP, purity: 98.7%		[redacted] 2001 (A3_1_1-01)
Odour at 20 °C and 101.3 kPA	-	Statement	No study was performed due to the inhalation hazard of the substance.	
	Odourless to almost odourless, any odour is faint and characteristic	Statement, non-GLP, purity 99.7%		[redacted] 2006 (A3.03.3_01)
Melting / freezing point	129 °C (decomposition at ca. 170 °C)	Directive 92/69/EEC, A.1, GLP, purity 99.7 g/100 g		[redacted] 2002 (A3.01.1_01)
	No melting point could be determined up to 150 °C (decomposition starts at about 160 °C)	Directive 92/69/EEC A.1 (DTA), GLP, purity: 98.7%	Results were confirmed by visual observations in a melt microscope. In the study of [redacted] (2001; A3_1_1-01), repeatedly two endothermal effects were observed in the DTA analysis at approx. 100 and 130 °C. However, they were not attributed to a melting process as confirmed visually by using a melting point microscope. From which endothermic process these observed endotherms result (like a phase transition other than melting, evaporation of impurities like residual	[redacted] 2001 (A3_1_1-01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			water, etc.) is not known. However, several other data sources indicate a melting point of Bronopol in the range of 130 °C, for instance: i.) Lide, D.R., G.W.A. Milne (eds.). Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. 1994., p. V4: 4326; ii.) US-EPA (https://archive.epa.gov/pesticides/reregistration/web/pdf/2770red.pdf) iii.) NTP, 1992 (referenced in: https://pubchem.ncbi.nlm.nih.gov/compound/Bronopol#section=Melting-Point) iv.) Studies/Information available at ECHA website (https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11419/4/3/?documentUUID=4b400757-7db4-41d5-af96-407038b1f160)	
Boiling point	There is no boiling point up to the decomposition of the test substance.	Directive 92/69/EEC A.2 (DTA), GLP, purity: 98.7%		[redacted] 2001 (A3_1_1-01)
	The normal boiling temperature cannot be determined.	Directive 92/69/EEC, A.2 and A.4, GLP, purity 99.7%	At pressures above 60 hPa temperatures decreased at constant pressures as a consequence of thermically caused changes in the test item.	[redacted] 2002a (A3.01.2_01)
Granulometry	Particle size distributions: D50 = 593–900 µm D10 = 305–519 µm D90 = 1010–1471 µm	CIPAC MT 187 non-GLP		[redacted] 2020 (no BPD-ID)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	Sample %-Through [in g/100 g] "Protectol BN": with sieve of mesh size [mm]: 0.630; 0.500; 0.355; 0.250; 0.180 Batch no. [redacted] 40; 23; 8; 3; 1 Batch no. [redacted] 38; 25; 10; 3; 1 Batch no. [redacted] 30; 18; 7; 3; 1 Batch no. [redacted] 38; 24; 10; 3; 1 Batch no. [redacted] 36; 22; 8; 3; 1 The estimated value of the measurement uncertainty is approx. +/- 26 % rel.	BASF internal standard method PM/00284, sieving method, purity min. 99.0%, GLP		[redacted] 2007 (B3.11_01)
	Particle size distributions: D50 = 683–830 µm D10 = 285–393 µm D90 = 1352–1470 µm	GMP, Method not specified		[redacted] 2007 (no BPD-ID)
Vapour pressure	$5.1*10^{-3}$ Pa at 20 °C $1*10^{-2}$ Pa at 25 °C	Directive 92/69/EEC, A.4, GLP, purity 99.7%	The values of the [redacted] study (2001; A3.01.3_01) and the [redacted] study (2002; A3_2-02) are almost identical for both given temperatures whereas in the study of [redacted] (2000a; A3_2-01) only limit values could be derived for those two temperatures. Because of the similarity of values, both, the [redacted] (2001, A3.01.3_01) and [redacted] (2002; A3_2-02) study were considered to be accurate, reasonable and reliable. The value of the vapour pressure determined in the [redacted] (2001; A3.01.3_01) study was used in the environmental risk assessment and is identical to the one listed in Appendix I: List	[redacted] 2001 (A3.01.3_01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			of endpoints. This study was preferred only because of the higher purity of the test item.	
	4.92*10^{-3} Pa at 20°C and 1.01*10^{-2} Pa at 25°C	Directive 92/69/EEC, A.4, OECD 104 OPPTS 830.7950 (Knudsen-Effusion weight loss method), GLP, purity: 98.6%	Measurements were performed in the temperature range from 10.23 °C to 41.20 °C.	[redacted] 2002 (A3_2-02)
	< 1*10^{-3} Pa at 20 °C < 1*10^{-3} Pa at 25 °C	Directive 92/69/EEC A.4 Vapour pressure balance, GLP, purity: 98.7%	For calculations, the Antoine equation and a melting point of 130 °C were used	[redacted] 2000a (A3_2-01)
Henry's law constant	1.16*10^{-6} Pa m^3/mol at 25 °C	Calculated with HENRYWIN v3.20 (Bond Contribution Method; EPI Suite v4.11).	Based on the Guidance on the BPR, Vol. I, Version 2.1, March 2022 and the therein referenced Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance Version 6.0, July 2017; the HLC calculation method based on water solubility, molecular weight and vapor pressure is only applicable to substances that have a low water solubility (i.e. < 1mol/L). Hence, the HLC was calculated using EPI Suite (bond method).	Estimation of the Henry's Law Constant HENRYWIN v3.20 (Bond Contribution Method; EPI Suite v4.11).
Surface tension	72 mN/m (at 20 °C and 1.0 g/L)	Directive 92/69/EEC, A.5, GLP, purity min. 99.0%	The test item is not surface-active.	[redacted] 2007 (A3.07_01)
	72.70 mN/m (at 19.9 °C and 1.0 g/L)	Directive 92/69/EEC A.5 (OECD harmonized ring method), GLP, purity: 98.7%	The test item is not surface-active.	[redacted] 2000b (A3_13-01)
Water solubility at 20 °C	pH temp. [°C] result 5 10.0 +/-0.5 248 +/- 40 g/L 5 20.0 +/-0.5 304 +/- 6 g/L 5 30.0 +/-0.5 360 +/- 25 g/L	OECD 105, flask method, HPLC-UV detection, GLP, purity 99.6%	The test item is not stable in water or at higher pH values. Therefore, the solubility was determined in buffer with a pH value of 5 with maximal equilibration times of 72 h. pH 5 was adjusted using a buffer consisting of Na_2HPO_4 and KH_2PO_4.	[redacted] 2021b (no BPD-ID)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			The value 304 g/L (pH 5; 20ºC) is used in the environmental risk assessment and is identical to the one listed in Appendix I: List of endpoints.	
	281.7 g/L (20 °C) at pH 3.5	Directive 92/69/EEC A.6 (flask method), GLP, purity: 98.7%	The determination of the water solubility at pH 7 (and above) is not possible. The test substance is not stable under these conditions and buffering the high concentrations of the test substance requires high concentrations of buffers. Interferences like salting-out are presumable.	[redacted] 2000a (A3_5-01)
Partition coefficient (*n*-octanol/water) and its pH dependency	log P_{OW}: –0.32 (at 10 °C and pH 3–4) –0.42 (at 20 °C and pH 3–4) –0.50 (at 30 °C and pH 3–4)	Directive 92/69/EEC A.8 OECD 107, purity 99.6 % (ratio of the solubilities in water and 1-octanol), GLP	Calculated with the solubilities in 1-octanol and in water. The determination of the log P_{OW} at pH 7 and pH 9 was not performed since the test substance is not stable under these conditions. The values of the studies of [redacted] (2021a; no BPD-ID), [redacted] (2000b; A3_9-01) and [redacted] (2007d; A3_9-02) are very close to each other and far away from critical in terms of the environmental risk assessment. The reason for taking the study of [redacted] (2007d; A3_9-02) as the key study is that the test item purity of the corresponding studies is the highest (99.6%) which is, however, also true for the study of [redacted] (2021a; no BPD-ID), but [redacted] (2007d; A3_9-02) furthermore provides information on the log p_{OW} at different temperatures. Therefore, the value(s) of the [redacted] (2007d; A3_9-02) study is used in the risk assessment and are given in Appendix I: List of endpoints.	[redacted] 2007d (A3_9-02)
	log P_{OW}: 0.15	OECD 107, shake flask	pH 4.9 was adjusted using a buffer	[redacted] 2021a

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	temperature: 23 °C pH: 4.9	method, HPLC-UV detection, GLP, purity 99.6%	consisting of Na_2HPO_4 and KH_2PO_4.	(no BPD-ID)
	log P_{OW} : 0.17 temperature: 23 °C pH: 4.0 ratios tested: 1:0.25; 1: 0.667 and 1:4	Directive 92/69/EEC A.8 (shake-flask method), GLP, purity: 98.7%	The determination of the log P_{OW} at pH 7 and pH 9 was not performed since the test substance is not stable under these conditions.	[redacted] 2000b (A3_9-01)
Thermal stability and identity of breakdown products	An exothermic process was observed starting at about 170 °C (decomposition). 1st reaction: Onset temperature: 155 °C Peak temperature: 218 °C Energy release: 2870 J/g	OECD Guideline 113, GLP, purity min. 99.0%		[redacted] 2007 (A3.10_01)
	During heating in a closed system an exothermic decomposition was observed starting at about 160 °C. In an open crucible (glass) volatilisation was observed between 145 °C and 185 °C. The residue of an ISTA-determination (closed system) was examined using FTIR-spectroscopy. No typical absorption bands of possible degradation products could be detected.	Directive 92/69/EEC A.1 (DTA and ISTA) (TG-FTIR for degradation products) GLP (FTIR analyses non-GLP), purity: 98.7%	The study was not done according to OECD 113. However, the study provides the relevant information, i.e. the substance is stable up to at least 150 °C, but volatilisation can start at 145 °C in open systems.	[redacted] 2001 (A3_1_1-01)
	Protectol BN is stable at normal temperatures, but when heated above 140 °C it decomposes exothermically liberating toxic hydrogen bromide and oxides of nitrogen and swelling up to give a sticky tarry mass which burns readily if	Statement based on experience in use and handling		[redacted] 2005 (A3.10_02)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	involved in a fire.			
Reactivity towards container material	Not expected	Statement based on experience in use, non GLP	Crystalline Bronopol (Myacide BT) in the dry state is not corrosive per se to metals and other packing materials. However, concentrated solutions of Bronopol are corrosive to a range of metals including mild steel, copper, brass and aluminium. These same solutions have been shown to be compatible with plastics used widely in packaging such as Low- and High-Density Polyethylene (LDPE and HDPE), Rigid PVC and Polypropylene.	[redacted] 2000 (A3.17_01)
	Not expected	Statement based on experience in use, non GLP	Based on experience in use, bronopol is not expected to react towards container material. Suitable container materials includes plastics widely used in packaging such as Low- and High-Density Polyethylene (LDPE and HDPE), Rigid PVC and Polypropylene. Unless moisture is present even contact with metals like aluminum would not lead to reactivity. Moreover, since Bronopol is very polar, direct absorption into elastomers or polymers would not be expected.	(no BPD-ID)
Dissociation constant	pK_a = 9.91 ± 0.36 at 20°C	OECD 112 (potentiometric titration), GLP, purity: 99.6%		[redacted] 2007b (A3_6-01)
	Titration with alkali gave a mean pK_a of 9.56 with standard deviation 0.04 and coefficient of variation of 0.4% (n=3).	USA EPA Pesticide Assessment Guidelines 63-10, GLP	The dissociation constant measured in this study was not a true pK_a of Bronopol since it degrades above pH 7.0 (Challis and Yousaf, 1991). The pK_a measured was likely to be a composite pK_a primarily associated with the initial decomposition product.	[redacted] 1991 (A3.06_01)
Viscosity	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Solubility in organic solvents, including effect of temperature on solubility	1-octanol: 90,300 mg/L at 10 °C 108,000 mg/L at 20 °C 133,000 g/L at 30 °C *n*-heptane: <15 mg/L at 10 °C 20 mg/L at 20 °C 34 mg/L at 30 °C acetone: >250,000 mg/L at 10 °C >250,000 mg/L at 20 °C >250,000 mg/L at 30 °C	CIPAC MT 157 CIPAC MT 181; GLP, purity: 99.6%	The solubility in acetone was determined visually.	[redacted] 2007c (A3_7-01)
	Solubility in methanol at 20 °C: 746,000 mg/L Solubility in methanol at 30 °C: 853,000 mg/L Solubility in toluene at 20 °C: 1,500 mg/L Solubility in toluene at 30 °C: 2,500 mg/L	Directive 92/69/EEC, A.6, GLP, purity min. 99.0 %		[redacted] 2007 (A3.07_01)
Stability in organic solvents used in biocidal products and identity of relevant degradation products	Not required.		The active substance as manufactured and the biocidal products do not contain organic solvents.	not applicable

A.1.3.1 Physical hazards and respective characteristics

Table A-7: Physical hazards and respective characteristics

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
Explosives	Recommendations on the Transport on Dangerous Goods, Manual of Tests and	Preventol P-100 Batch: [redacted] Purity 99.9 %	Test Series 2 2 (a) UN gap test,	[redacted] 2015 (no BPD-ID)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
	Criteria (Rev 5, 2009) UN Test Series 2: 2(a) UN gap test 2(b) Koenen test 2(c) Time/pressure test Test F.3 BAM Trauzl test	GLP: No	Result: negative. The tube was not dismantled. No hole in the plate. 2 (b) Koenen test, Result: negative. Limiting diameter < 1.0 mm. According the UN-MTC, the substance does not show violent effect on heating under confinement if the limiting diameter is less than 2.0 mm. 2 (c) Time/pressure test Result: negative. Maximum pressure reached: 1031 kPa, 719 kPa, 1063 kPa. The substance shows no deflagration because a gauche pressure of 2070 kPa is not reached in any of the three tests. Bronopol is too insensitive for inclusion in Class I (explosives). Test F.3 BAM Trauzl test was 'not low' (i.e. expansion of the lead block is > 25 cm^3/10g of sample).	
	Directive 92/69/EEC A.14	Protectol BN Batch: [redacted] Purity min. 99.0 % GLP: Yes	The test substance is not considered to exhibit a danger of explosion in the sense of the EEC Guideline A.14. a) Thermal sensitivity Steel sleeve test: Limiting diameter 2 and 6 mm → no explosion b) Mechanical sensitivity (shock) Falling weight test: Weight 10 kg, height 0.4 m → no explosion c) Mechanical sensitivity (friction)	[redacted] 2007 (A3.10_01)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			Friction test: No explosion, no crepitation and no flames	
	Directive 92/69/EEC A.14	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	The thermal sensitivity (Koenen test), the mechanical sensitivity (BAM Drop-Weight Test and BAM friction mill) as outlined in the EEC Guideline A.14 were tested. Negative results were obtained in the three tests. No fragments observed (cartridge unchanged) in the Koenen test and no optical changes observed in the mechanical sensitivity test. The test substance is not explosive in the sense of the EEC Guideline A.14	[redacted] 2000 (A3_11-01)
	Recommendations on the Transport of Dangerous Goods, Test and Criteria, United Nations, 1986, Parts I + II	Bronopol Purity: 99.8% GLP: No	Test Series 1: • Test 1 (a) BAM 50/60 steel tube test: propagation of detonation Result: positive. Propagation of detonative reaction. Tube completely fragmented into long strips. • Test 1 (b) Koenen test: thermal response Result: positive. Exhibits thermal explosive properties. Limiting diameter 1.0 mm (a limiting diameter of 1.0 mm or above is considered positive) Test Series 2: • Test 2 (a) BAM 50/60 steel tube test: sensitivity to schock	[redacted] 1992 (A3.11_02)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			Result: negative. No propagation of detonative reaction. • Test 2 (b) Koenen test: thermal sensitivity Result: negative. Thermally too insensitive for inclusion in Class 1 (explosives). Limiting diameter 1.0 mm (in test series 2, a limiting diameter < 2.0 mm is considered a negative result) • Test 2 (c) (i) Time/pressure test Result: negative (because the maximum pressure of 2070 kPa gauche is not reached). Maximum pressure reached: 4 bar (400 kPa), Thermally too insensitive for inclusion in Class 1 (explosives) <u>Test Series 3</u> • Test 3 (a) (ii) BAM Fallhammer: Limiting impact energy > 40 J Result: negative, as the limiting impact energy is > 2 J. • Test 3 (b) (i) BAM friction apparatus: Limiting load > 363 N Result: negative. According to the UN-MTC, *the test result is considered positive if the lowest friction load at which one "explosion" occurs in six trials is less than 80 N* […]. *Otherwise, the test result is considered negative*.	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			Therefore, the test result is negative and the substance is insensitive to friction stimuli. Conclusion: These tests indicate that bronopol is relatively insensitive to initiation by friction or impact. Application of the Test Series 1 of the UN-MTC Class 1 (explosives) acceptance procedure indicated that propagation of detonative reaction can occur and that the substance exhibits some thermal explosive properties. However, the results from Test Series 2 indicate that bronopol is too insensitive for acceptance into Class 1 (explosives).	
Flammable gases	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Flammable aerosols	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Oxidising gases	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Gases under pressure	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Flammable liquids	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
Flammable solids	Directive 92/69/EEC A.10	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	The preliminary test was negative. Local burning followed by rapid extinction was observed. The main test was omitted due to the result of the preliminary test. The test substance is not considered highly flammable	[redacted] 2007 (A3.10_01)
	Directive 92/69/EEC A.10	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	The test substance melted when approached by the ignition flame. The substance did not burn down or burn up. The test substance is not considered highly flammable	[redacted] 2000 (A3_11-01)
Self-reactive substances and mixtures	UN H.4	Preventol P-100 Batch: [redacted] Purity: 99.6% GLP: No	SADT (self-accelerating decomposition temperature) > 75 °C No exothermic effects were recorded. A SADT above 75 °C is an exclusion criterion for classification in this hazard class.	[redacted] 2011 (no BPD-ID)
Pyrophoric liquids	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Pyrophoric solids	Expert Statement		The substance is a solid and based on many years experience of handling bronopol, it can be confirmed that the substance does not spontaneously ignite in air.	[redacted] 2007a (A3.11/03)
Self-heating substances and mixtures	Directive 92/69/EEC A.16	Protectol BN (Batch identification: [redacted]), purity min. 99.0%, GLP	No self-heating detected up to 400ºC.	[redacted] 2007 (A3.10_01)
	Directive 92/69/EEC A.16	Bronopol Bayer Batch: [redacted] Purity: 98.7%, GLP	No exothermic effects were recorded. The substance melted during the test. Bronopol does not undergo	[redacted] 2000 (A3_11-01)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			spontaneous combustion according to EEC Guideline A.16.	
Substances and mixtures which in contact with water emit flammable gases	Expert Statement		The chemical structure of the substance does not contain metals or metalloids. It can be concluded that the test substance does not liberate flammable gases in hazardous amounts upon contact with water.	[redacted] 2006 (A3_11-02)
	Expert Statement		The substance is a solid and based on many years experience of handling bronopol, it can be confirmed that the substance is not flammable in water.	[redacted] 2007a (A3.11/03)
Oxidising liquids	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Oxidising solids	Directive 92/69/EEC A.17	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture. Burning rate of the mixtures tested: The highest burning rate of 2.22 mm/s was determined with a mixture containing 10% weight of test substance. Burning rate of the reference mixture: The highest burning rate of 5.0 mm/s was determined with a barium nitrate/cellulose mixture containing 60% weight of oxidiser.	[redacted] 2007 (A3.10_01)
	Directive 92/69/EEC A.17	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested (0.7 mm/s in a mixture 10 % substance/ 90 % cellulose) is lower than the maximum burning rate of the	[redacted] 2000 (A3_11-01)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
			reference mixture (1.1 mm/s) Therefore, bronopol is not an oxidiser.	
Organic peroxides	Not applicable	Not applicable	Waiver: The active substance contains no R-O-O-R peroxide group in its chemical structure. Thus, the product must not be classified as organic peroxides and testing is not required.	
Corrosive to metals	Not applicable	Not applicable	Waiver: According to the ECHA Guidance on the Application of the CLP Criteria, Section 2.16 Corrosive to metals, and the UN Test C.1 Guideline, only liquids and solids that may become liquid must be tested for this endpoint. Since the active substance is solid and has a melting point > 55 °C, testing and classification is not necessary. However, concentrated solutions of Bronopol are corrosive to a range of metals including mild steel, copper, brass and aluminium (see Table A-9; 'Reactivity towards container material').	
Desensitised explosives	Not applicable	Not applicable	Waiver: Since the active substance is not considered as an explosive substance or mixture, classification and testing is scientifically not necessary and this endpoint can be waived accordingly.	
Auto-ignition temperature (liquids and gases)	Not applicable	Not applicable	Waiver: As the active substance is solid, testing is scientifically not necessary and this endpoint can be waived accordingly.	
Relative self-ignition temperature for solids	Directive 92/69/EEC A.16	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	Not a readily combustible solid, the substance melted during the test. Bronopol does not undergo spontaneous combustion according to EEC Guideline A.16.	[redacted] 2000 (A3_11-01)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
	Directive 92/69/EEC A.16	Protectol BN (Batch identification: [redacted]), purity min. 99.0%, GLP	No self-heating detected up to 400°C.	[redacted] 2007 (A3.10_01)
Dust explosion hazard	VDI Guideline 2263, Part 1 DIN EN 14034-1-3:2011 DIN EN ISO 80079-20-2:2016-12	Protectol BN Batch: [redacted] Purity: 99.6% GLP: Yes	Dust explosibility in the Hartmann tube: substance could not be ignited (d_{50} = 26 µm); Therefore, the minimum ignition energy is > 10 J. Lower explosion limit in the 20 L-sphere (d_{50} = 26 µm): 250 g/m³. The dust has to be considered as ignitable (DIN EN 14034, Part 3). Dust explosion characteristics in the 20 L-sphere (d_{50} = 26 µm): maximum explosion pressure = 5.9 $bar_{g;}$ maximum pressure increase rate dp/dt = 188 bar/s. Derived from the K_{St}-value of 51 bar*m/s, the grounded and sieved **test item is proposed to be classified in dust explosion class St 1** (DIN EN 14034, Part 1 and 2). Minimum ignition energy (MIE): not performed according to results of the Hartmann tube test. Minimum ignition temperature of a dust cloud (MIT): 800 °C (Godbert-Greenwald oven; d_{50} = 26 µm; DIN EN ISO 80079-20-2) Minimum ignition temperature of a dust layer (smouldering temperature): no smouldering temperature exists due to the fact that the test item melts before a smouldering fire occurs.	[redacted] 2020a (no BPD-ID)

A.1.3.2 Assessment of physical hazards according to the CLP criteria

Not applicable for the CLH report.

A.1.3.3 Explosives

Table A-8: Summary table of studies on explosive properties*

Method	Results	Remarks	Reference
Recommendations on the Transport on Dangerous Goods, Manual of Tests and Criteria (Rev 5, 2009) UN Test Series 2: 2 (a) UN gap test 2 (b) Koenen test 2 (c) Time/pressure test Test F.3 BAM Trauzl test	Test Series 2 2 (a) UN gap test, Result: negative. The tube was not dismantled. No hole in the plate. 2 (b) Koenen test, Result: negative. Limiting diameter < 1.0 mm. According the UN-MTC, the substance does not show violent effect on heating under confinement if the limiting diameter is less than 2.0 mm. 2 (c) Time/pressure test Result: negative. Maximum pressure reached: 1031 kPa, 719 kPa, 1063 kPa. The substance shows no deflagration because a gauche pressure of 2070 kPa is not reached in any of the three tests. Bronopol is too insensitive for inclusion in Class I (explosives). Test F.3 BAM Trauzl test was 'not low' (i.e. expansion of the lead block is > 25 cm^3/10g of sample).	Preventol P-100 Batch: [redacted] Purity 99.9 % GLP: No	[redacted] 2015 (no BPD-ID)
Directive 92/69/EEC A.14	The test substance is not considered to exhibit a danger of explosion in the sense of the EEC Guideline A.14.	Protectol BN Batch: [redacted] Purity min. 99.0 % GLP: Yes	[redacted] 2007 (A3.10_01)

	a) Thermal sensitivity Steel sleeve test: Limiting diameter 2 and 6 mm → no explosion b) Mechanical sensitivity (shock) Falling weight test: Weight 10 kg, height 0.4 m → no explosion c) Mechanical sensitivity (friction) Friction test: No explosion, no crepitation and no flames		
Directive 92/69/EEC A.14	The thermal sensitivity (Koenen test), the mechanical sensitivity (BAM Drop-Weight Test and BAM friction mill) as outlined in the EEC Guideline A.14 were tested. Negative results were obtained in the three tests. No fragments observed (cartridge unchanged) in the Koenen test and no optical changes observed in the mechanical sensitivity test. The test substance is not explosive in the sense of the EEC Guideline A.14	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)
Recommendations on the Transport of Dangerous Goods, Test and Criteria, United Nations, 1986, Parts I + II	Test Series 1: • Test 1 (a) BAM 50/60 steel tube test: propagation of detonation Result: positive. Propagation of detonative reaction. Tube completely fragmented into long strips.	Bronopol Purity: 99.8% GLP: No	[redacted] 1992 (A3.11_02)

	• Test 1 (b) Koenen test: thermal response Result: positive. Exhibits thermal explosive properties. Limiting diameter 1.0 mm (a limiting diameter of 1.0 mm or above is considered positive) <u>Test Series 2</u>: • Test 2 (a) BAM 50/60 steel tube test: sensitivity to schock Result: negative. No propagation of detonative reaction. • Test 2 (b) Koenen test: thermal sensitivity Result: negative. Thermally too insensitive for inclusion in Class 1 (explosives). Limiting diameter 1.0 mm (in test series 2, a limiting diameter < 2.0 mm is considered a negative result) • Test 2 (c) (i) Time/pressure test Result: negative (because the maximum pressure of 2070 kPa gauche is not reached). Maximum pressure reached: 4 bar (400 kPa), Thermally too insensitive for inclusion in Class 1 (explosives) <u>Test Series 3</u> • Test 3 (a) (ii) BAM Fallhammer: Limiting impact energy > 40 J		

	Result: negative, as the limiting impact energy is > 2 J. • Test 3 (b) (i) BAM friction apparatus: Limiting load > 363 N Result: negative. According to the UN-MTC, *the test result is considered positive if the lowest friction load at which one "explosion" occurs in six trials is less than 80 N* […]. *Otherwise, the test result is considered negative*. Therefore, the test result is negative and the substance is insensitive to friction stimuli. Conclusion: These tests indicate that bronopol is relatively insensitive to initiation by friction or impact. Application of the Test Series 1 of the UN-MTC Class 1 (explosives) acceptance procedure indicated that propagation of detonative reaction can occur and that the substance exhibits some thermal explosive properties. However, the results from Test Series 2 indicate that <u>bronopol is too insensitive for acceptance into Class 1 (explosives)</u>.		
UN H.4 (Heat accumulation storage test)	SADT (self-accelerating decomposition temperature) > 75°C No exothermic effects were recorded.	Preventol P-100 Batch: [redacted] Purity: 99.6% GLP: No	[redacted] 2011 (no BPD-ID)

	A SADT above 75 °C is an exclusion criterion for classification in this hazard class. (Self-reactive substances and mixtures)		
Directive 92/69/EEC A.16 (Relative self-ignition temperature for solids)	No self-heating detected up to 400 °C. (Self-heating substances and mixtures)	Protectol BN (Batch identification: [redacted]), purity min. 99.0%, GLP	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.16 (Relative self-ignition temperature for solids)	No exothermic effects were recorded. The substance melted during the test. Bronopol does not undergo spontaneous combustion according to EEC Guideline A.16. (Self-heating substances and mixtures/Relative self-ignition temperature for solids)	Bronopol Bayer Batch: [redacted] Purity: 98.7%, GLP: yes	[redacted] 2000 (A3_11-01)
Directive 92/69/EEC A.10 (Flammability, solids)	The preliminary test was negative. Local burning followed by rapid extinction was observed. The main test was omitted due to the result of the preliminary test. The test substance is not considered highly flammable	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.10 (Flammability, solids)	The test substance melted when approached by the ignition flame. The substance did not burn down or burn up. The test substance is not considered highly flammable.	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)
Expert Statement (Pyrophoric properties of solids	The substance is a solid and based on many years experience of handling bronopol, it can be		[redacted] 2007a (A3.11/03)

and liquids)	confirmed that the substance does not spontaneously ignite in air.		
Directive 92/69/EEC A.17 (Oxidising properties, solids)	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture. Burning rate of the mixtures tested: The highest burning rate of 2.22 mm/s was determined with a mixture containing 10% weight of test substance. Burning rate of the reference mixture: The highest burning rate of 5.0 mm/s was determined with a barium nitrate/cellulose mixture containing 60% weight of oxidiser.	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.17 (Oxidising properties, solids)	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested (0.7 mm/s in a mixture 10 % substance/ 90 % cellulose) is lower than the maximum burning rate of the reference mixture (1.1 mm/s) Therefore, bronopol is not an oxidiser.	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)

**Include additional details below the table and/or a reference to the Appendix VII containing the study summaries, if relevant.*

A.1.3.3.1 Short summary and overall relevance of the provided information on explosive properties

The active substance was tested according UN test series 2 (UN RTDG, Manual of Tests and Criteria; Part I, Classification procedures, test methods and criteria relating to explosives, seventh revised edition, 2019) and according to Directive 92/69/EEC A.14 for thermal sensitivity, mechanical sensitivity (shock) and mechanical sensitivity (friction). In none of those tests explosive properties were detected in the sense of the directive. In one study ([redacted] 1992; A3.11_02) it was concluded that "the substance was found to be detonable and exhibited some thermal explosive properties according to UN Test Series 1 but according to UN Test Series 2 bronopol is too insensitive for inclusion in Class 1 (explosives) by the United Nations Class 1 acceptance scheme". The same conclusion is provided by the study of [redacted] (2015; no BPD-ID) and [redacted] (2011; no BPD-ID), i.e. too insensitive for acceptance in class 1. Furthermore, test UN H.4 was conducted whereby a SADT (self-accelerating decomposition temperature) > 75°C and no exothermic effects were recorded. Tests according to directive 92/69/EEC method A.17, A.16 and A.10 were performed in which no oxidising properties, no self-heating up to 400 °C, no spontaneous combustion and no highly flammable properties were detected. Eventually, the active substance is known to be not a pyrophoric solid. The result of the BAM Trauzl test (UN F.3) was 'not low' (i.e. expansion of the lead block is > 25 cm^3/10g of sample).

A.1.3.3.2 Comparison with the CLP criteria

As described above, according to the three negative results of UN Test series 2, the substance is considered too insensitive for acceptance in class 1. One test of each test series 1 to 3 described in the UN RTDG, Manual of Tests and Criteria (Part I, Classification procedures, test methods and criteria relating to explosives, seventh revised edition, 2019), and according to Directive 92/69/EEC (method A.14.) was performed. These include a test for thermal sensitivity (Koenen Test, UN Test Series 1 (b) and Test Series 2(b)), mechanical sensitivity (shock; BAM Fallhammer, UN Test Series 3 (a) (ii)) and mechanical sensitivity (friction; BAM friction mill; UN Test Series 3 (b) (i)). In none of these tests, explosive properties of the active substance were observed that would lead to a classification according to the criteria set out in the CLP regulation (Regulation EC 1272/2008).

A.1.3.3.3 Conclusion on classification and labelling for explosive properties

No classification needed.

However, due to the 'not low' result of the BAM Trauzl test, it is recommended within the scope of the CLP Regulation to communicate this result to users by inclusion of the label EUH044 – 'Risk of explosion if heated under confinement'.

A.1.3.4 Flammable gases (including chemically unstable gases)

No data available.

A.1.3.4.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

No data available.

A.1.3.4.2 Comparison with the CLP criteria

As the active substance is not a gas, this endpoint is not applicable.

A.1.3.4.3 Conclusion on classification and labelling for flammable gases

No classification needed.

A.1.3.5 Flammable aerosols and aerosols

No data available.

A.1.3.5.1 Short summary and overall relevance of the provided information on flammable aerosols and aerosols

No data available.

A.1.3.5.2 Comparison with the CLP criteria

As the active substance is not an aerosol, this endpoint is not applicable.

A.1.3.5.3 Conclusion on classification and labelling for flammable aerosols and aerosols

No classification needed.

A.1.3.6 Oxidising gases

No data available.

A.1.3.6.1 Short summary and overall relevance of the provided information on oxidising gases

No data available.

A.1.3.6.2 Comparison with the CLP criteria

As the active substance is not a gas, this endpoint is not applicable.

A.1.3.6.3 Conclusion on classification and labelling for oxidising gases

No classification needed.

A.1.3.7 Gases under pressure

No data available.

A.1.3.7.1 Short summary and overall relevance of the provided information on gases under pressure

No data available.

A.1.3.7.2 Comparison with the CLP criteria

As the active substance is not a gas, this endpoint is not applicable.

A.1.3.7.3 Conclusion on classification and labelling for gases under pressure

No classification needed.

A.1.3.7.4 Flammable liquids

No data available.

A.1.3.7.5 Short summary and overall relevance of the provided information on flammable liquids

No data available.

A.1.3.7.6 Comparison with the CLP criteria

As the active substance is not a liquid, this endpoint is not applicable.

A.1.3.7.7 Conclusion on classification and labelling for flammable liquids

No classification needed.

A.1.3.8 Flammable solids

Table A-9: Summary table of studies on flammable solids*

Method	Results	Remarks	Reference
Directive 92/69/EEC A.10 (Flammability, solids)	The preliminary test was negative. Local burning followed by rapid extinction was observed. The main test was omitted due to the result of the preliminary test. The test substance is not considered highly flammable.	The applied test method is basically identical to the screening procedure described in Part III, Sub-section 33.2.4.3.1 in the UN-MTC which is recommended as screening test for this endpoint in the Guidance on the Application of the CLP Criteria (section 2.7.4.2, Version 5.0, July 2017).	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.10 (Flammability, solids)	The test substance melted when approached by the ignition flame. The substance did not burn down or burn up. The test substance is not considered highly flammable.	The applied test method is basically identical to the screening procedure described in Part III, Sub-section 33.2.4.3.1 in the UN-MTC which is recommended as screening test for this endpoint in the Guidance on the Application of the CLP Criteria (section 2.7.4.2, Version 5.0, July 2017).	[redacted] 2000 (A3_11-01)

A.1.3.8.1 Short summary and overall relevance of the provided information on flammable solids

The active substance was tested according to Directive 92/69/EEC Method A.10. This test method is basically identical to the screening procedure described in Part III, Sub-section 33.2.4.3.1 in the UN-MTC which is recommended as screening test for this endpoint in the Guidance on the Application of the CLP Criteria (section 2.7.4.2, Version 5.0, July 2017).

A.1.3.8.2 Comparison with the CLP criteria

As the active substance was consistently found to be not a flammable solid in the sense of the applied method, no classification is needed for this endpoint as stated in section 2.7.4.5. (Decision logic) of the Guidance on the Application of the CLP Criteria (section 2.7.4.2, Version 5.0, July 2017).

A.1.3.8.3 Conclusion on classification and labelling for flammable solids

No classification needed.

A.1.3.8.4 Self-reactive substances

Table A-10: Summary table of studies on self-reactivity*

Method	Results	Remarks	Reference
UN H.4 (Heat accumulation storage test)	SADT (self-accelerating decomposition temperature) > 75 °C No exothermic effects were recorded. A SADT above 75 °C is an exclusion criterion for classification in this hazard class.		[redacted] 2011 (no BPD-ID)

A.1.3.8.5 Short summary and overall relevance of the provided information on self-reactive substances

A test according to UN H.4 (Heat accumulation storage test) was performed which revealed a SADT (self-accelerating decomposition temperature) of > 75 °C and no exothermic effects were recorded.

A.1.3.8.6 Comparison with the CLP criteria

The SADT (self-accelerating decomposition temperature) is > 75 °C and no exothermic effects were recorded in test UN H.4 (Heat accumulation storage test). A SADT above 75 °C is an exclusion criterion for classification in this hazard class according to section 2.8.4.2 of the Guidance on the Application of the CLP Criteria (Version 5.0, July 2017) and section 2.8.2.1 (e) of the CLP Regulation (Regulation EC 1272/2008). Therefore, the classification criteria as given in Chapter 2.8.4.2 of the CLP Guidance are not met.

A.1.3.8.7 Conclusion on classification and labelling for self-reactive substances

No classification needed.

A.1.3.9 Pyrophoric liquids

No data available.

A.1.3.9.1 Short summary and overall relevance of the provided information on pyrophoric liquids

No data available.

A.1.3.9.2 Comparison with the CLP criteria

As the active substance is not a liquid, this endpoint is not applicable.

A.1.3.9.3 Conclusion on classification and labelling for pyrophoric liquids

No classification needed.

A.1.3.10 Pyrophoric solids

Table A-11: Summary table of studies on pyrophoric solids*

Method	Results	Remarks	Reference
Expert Statement (Pyrophoric properties of solids and liquids)	The substance is a solid and based on many years experience of handling bronopol, it can be confirmed that the substance does not spontaneously ignite in air.		[redacted] 2007a (A3.11/03)
Directive 92/69/EEC A.16 (Self-heating substances and mixtures)	No self-heating detected up to 400 °C.	Protectol BN (Batch identification: [redacted]), purity min. 99.0%, GLP	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.16 (Self-heating substances and mixtures)	No exothermic effects were recorded. The substance melted during the test. Bronopol does not undergo spontaneous combustion according to EEC Guideline A.16.	Bronopol Bayer Batch: [redacted] Purity: 98.7%, GLP	[redacted] 2000 (A3_11-01)
Expert Statement (Substances and mixtures which in contact with water emit flammable gases)	The chemical structure of the substance does not contain metals or metalloids. It can be concluded that the test substance does not liberate flammable gases in hazardous amounts upon contact with water.		[redacted] 2006 (A3_11-02)

Expert Statement (Substances and mixtures which in contact with water emit flammable gases)	The substance is a solid and based on many years experience of handling bronopol, it can be confirmed that the substance is not flammable in water.		[redacted] 2007a (A3.11/03)
Directive 92/69/EEC A.10 (Flammable solids)	The preliminary test was negative. Local burning followed by rapid extinction was observed. The main test was omitted due to the result of the preliminary test. The test substance is not considered highly flammable.	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.10 (Flammable solids)	The test substance melted when approached by the ignition flame. The substance did not burn down or burn up. The test substance is not considered highly flammable.	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)

A.1.3.10.1 Short summary and overall relevance of the provided information on pyrophoric solids

Based on the experience in manufacture and handling, the active substance does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time (days)) and therefore has no pyrophoric properties. Furthermore, the substance is not self-heating, nor considered a flammable solid or emits flammable gases in contact with water.

A.1.3.10.2 Comparison with the CLP criteria

Based on the criteria outlined in the CLP regulation, the classification procedure for pyrophoric solids needs not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance or mixture is known to be stable at room temperature for prolonged periods of time (days)). Bronopol has been handled extensively in air and has never self-ignited. As the active substance shows furthermore no self-heating up to 400 °C in larger amounts, it is considered to be not a flammable solid and emits no flammable gases in contact with water, it can be concluded that also pyrophoric properties are absent and testing according to UN Test N.2 is not needed.

A.1.3.10.3 Conclusion on classification and labelling for pyrophoric solids

No classification needed.

A.1.3.11 Self-heating substances

Table A-12: Summary table of studies on self-heating substances*

Method	Results	Remarks	Reference
Directive 92/69/EEC A.16 (Self-heating substances and mixtures)	No self-heating detected up to 400 °C.	Protectol BN (Batch identification: [redacted]), purity min. 99.0%, GLP	[redacted] 2007 (A3.10_01)
Directive 92/69/EEC A.16 (Self-heating substances and mixtures)	No exothermic effects were recorded. The substance melted during the test. Bronopol does not undergo spontaneous combustion according to EEC Guideline A.16.	Bronopol Bayer Batch: [redacted] Purity: 98.7%, GLP	[redacted] 2000 (A3_11-01)
Directive 92/69/EEC A.1 (Melting / freezing point)	129 °C (decomposition at ca. 170 °C)	GLP, purity 99.7%	[redacted] 2002 (A3.01.1_01)

A.1.3.11.1 Short summary and overall relevance of the provided information on self-heating substances

Two tests according to method A.16 (Self-heating substances and mixtures) were performed and the melting point of the active substance was determined.

A.1.3.11.2 Comparison with the CLP criteria

Test according to UN Test N.4 as described in Section 33.3.1.6 of the UN-MTC should be applied to address this endpoint. Although the CLP Guidance states that “EU test method A.16 as described in Regulation (EC) No 440/2008 checks for self-heating properties. However, the method used is generally inappropriate for a sound assessment, and the findings do not lead to a classification. Therefore, special care must be taken if results from EU test method A.16 are interpreted towards a CLP classification for self-heating substances and mixtures”. Nevertheless, if according to EU test method A.16, no effects were observed, this can be used as an exclusion criterion as the test is done up to 400 °C and if there are no effects up to this temperature, the outcome of a test according to UN Test N.4 will be also negative. In addition, the guidance further specifies that “Substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced. However, this criterion is only applicable if the substance or mixture is completely molten up to this temperature”.
As the melting point of the active substance is < 160 °C, bronopol is not considered for classification in this class.

A.1.3.11.3 Conclusion on classification and labelling for self-heating substances

No classification needed.

A.1.3.12 Substances which in contact with water emit flammable gases

Table A-13: Summary table of studies on substances which in contact with water emit flammable gases*

Method	Results	Remarks	Reference
Expert Statement (Substances which in contact with water emit flammable gases)	The chemical structure of the substance does not contain metals or metalloids. It can be concluded that the test substance does not liberate flammable gases in hazardous amounts upon contact with water.		[redacted] 2006 (A3_11-02)
Expert Statement (Substances which in contact with water emit flammable gases)	The substance is a solid and based on many years experience of handling bronopol it can be confirmed that the substance is not flammable in water, does not spontaneously ignite in air.		[redacted] 2007a (A3.11/03)

A.1.3.12.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Expert statements are available which point out that from the structural formula of Bronopol and experience in use, it can be concluded that the test substance does not liberate flammable gases in hazardous amounts upon contact with water or humidity.

A.1.3.12.2 Comparison with the CLP criteria

As stated in section 2.12.4.2 of the ECHA guidance on the Application of the CLP Criteria (version 5.0, July 2017), and in section A6.5.4 of the Appendix 6 of the UN-MTC (revision 7, 2019), the classification procedure for substances which in contact with water may react to emit flammable gases need not to be applied if one of the following conditions is matched:

- the chemical structure of the substance does not contain metals or metalloids; or
- experience in production or handling shows that the substance does not react with water, e.g., the substance is manufactured with water or washed with water; or
- the substance is known to be soluble in water to form a stable mixture.

For the active substance, at least one of these conditions is fully met (does not contain metals or metalloids). Therefore, classification as substance which in contact with water emit flammable gases would not be applicable and thus testing would not be required. In addition, from the experience in use, it can be concluded that the active substance can therefore reasonably be expected not to be a substance which

in contact with water emits flammable gases. Thus, the study is scientifically not justified, and this endpoint can be waived accordingly.

A.1.3.12.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification needed.

A.1.3.13 Oxidising liquids

No data available.

A.1.3.13.1 Short summary and overall relevance of the provided information on oxidising liquids

No data available.

A.1.3.13.2 Comparison with the CLP criteria

As the active substance is not a liquid, this endpoint is not appliable.

A.1.3.13.3 Conclusion on classification and labelling for oxidising liquids

No classification needed.

A.1.3.14 Oxidising solids

Table A-14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Directive 92/69/EEC A.17 (Oxidising properties, solids)	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture. Burning rate of the mixtures tested: The highest burning rate of 2.22 mm/s was determined with a mixture containing 10% weight of test substance. Burning rate of the reference mixture: The highest burning rate of 5.0 mm/s was determined	Protectol BN Batch: [redacted] Purity min. 99.0% GLP: Yes	[redacted] 2007 (A3.10_01)

	with a barium nitrate/cellulose mixture containing 60% weight of oxidiser.		
Directive 92/69/EEC A.17 (Oxidising properties, solids)	The test substance is not considered an oxidising substance because the maximum burning rate of the mixtures tested (0.7 mm/s in a mixture 10 % substance/ 90 % cellulose) is lower than the maximum burning rate of the reference mixture (1.1 mm/s) Therefore, bronopol is not an oxidiser.	Bronopol Bayer Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)

A.1.3.14.1 Short summary and overall relevance of the provided information on oxidising solids

Tests on oxidising properties for solids according to Directive 92/69/EEC A.17 (horizontal burn rate test) were performed.

A.1.3.14.2 Comparison with the CLP criteria

According to the CLP regulation, a test according to UN Test O.1 as described in Section 34.4.1 of the UN-MTC is required. However, test according to Directive 92/69/EEC, method A.17 gives similar information about oxidising properties of solids. As the active substance is negative in two A.17 tests, it is concluded that the substance has no oxidising properties.

A.1.3.14.3 Conclusion on classification and labelling for oxidising solids

No classification needed.

A.1.3.15 Organic peroxides

No data available.

A.1.3.15.1 Short summary and overall relevance of the provided information on organic peroxides

No data available.

A.1.3.15.2 Comparison with the CLP criteria

As outlined in CLP Annex I, 2.15.4.1, organic peroxides are classified by definition based on their chemical structure. The active substance contains no R-O-O-R peroxide group in its chemical structure. Thus, the substance must not be classified as organic peroxide and testing is not required.

A.1.3.15.3 Conclusion on classification and labelling for organic peroxides

No classification needed.

A.1.3.16 Corrosive to metals

No data available.

A.1.3.16.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data available.

A.1.3.16.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (section 2.16, Version 5.0, July 2017), and the UN Test C.1 manual (UN-MTC, revision 7, 2019), only liquids and solids that may become liquid must be tested for this endpoint. Since the active substance is solid and has a melting point > 55 °C, testing and classification is not necessary.

A.1.3.16.3 Conclusion on classification and labelling for corrosive to metals

No classification needed.

A.1.3.17 Desensitised explosives

See chapter A.1.3.3 for further information.

A.1.3.17.1 Short summary and overall relevance of the provided information on the hazard class desensitised explosives

See chapter A.1.3.3 for further information.

A.1.3.17.2 Comparison with the CLP criteria

Since the active substance is not considered as an explosive substance (see chapter A.1.3.3 for further information), and the substance is not phlegmatised, classification and testing is scientifically not necessary and this endpoint can be waived accordingly.

A.1.3.17.3 Conclusion on classification and labelling for desensitised explosives

No classification needed.
However, due to the ‘not low’ result of the BAM Trauzl test, it is recommended within the scope of the CLP Regulation to communicate this result to users by inclusion of the label EUH044 – ‘Risk of explosion if heated under confinement’.

A.1.4 Analytical methods for detection and identification

Not applicable for the CLH report.

A.2 Effects against target organisms

Not applicable for the CLH report.

A.2.1 Intended uses

Not applicable for the CLH report.

Table A-15: Summary table of intended uses

Summary table of intended use(s)	
Product Type	2 (Intended Use: Disinfection of chemical toilets)
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Thereby, thiol-groups are catalytically oxidised to disulphide bonds with rapid consumption of oxygen.
Instruction for use	The sanitary additives together with a certain amount of water (depending on the actual product and the size of the respective tank) are filled into the sewage tank of the chemical toilet as so-called pre-charge.

Summary table of intended use(s)	
Product Type	6 (Intended Use: in-can preservatives of detergents and cleaning products)
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Thereby, thiol-groups are catalytically oxidised to disulphide bonds with rapid consumption of oxygen.
Instruction for use	During the formulation of the preserved product (e.g. surface cleaner), the biocidal product is homogenously incorporated into the end-product. In some cases, the biocidal product may be diluted prior to its incorporation.

Summary table of intended use(s)	
Product Type	11 (Intended Use: Preservatives for liquid-cooling and processing systems)
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Thereby, thiol-groups are catalytically oxidised to disulphide bonds with rapid consumption of oxygen.
Instruction for use	The biocidal product may be applied directly or, alternatively, as pre-mix into the water matrix to be preserved. A homogenous incorporation of the active substance into the system being treated is to be ensured.

Summary table of intended use(s)	
Product Type	12 (Intended Use: slimicide, paper industry)
Mode of action	Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Thereby, thiol-groups are catalytically oxidised to disulphide bonds with rapid consumption of oxygen.
Instruction for use	The biocidal product may be applied directly or, alternatively, as pre-mix into the water circuit to be preserved – ideally to the primary white water circuit. A homogenous incorporation of the active substance into the system being treated is to be ensured.

A.2.2 Summary on efficacy

A.2.2.1 Efficacy

Table A-16: Experimental data on the efficacy of the active substance against target organism(s)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
Bactericidal	PT 2,6, 11 and 12	BIOBAN BP-Plus: 100% Bronopol	*Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	Inhouse method from Dow Microbiocides Laboratory: Minimum Cidal Concentration (MCC) test.	Bronopol was manually pipetted to a pH-buffered solution containing the bacteria. After incubation at 37ºC the MCC was determined. Concentrations: Blanks without a.s, 5, 10, 20, 40 and 80 ppm. Exposure: 24 hours. The number of replicates is no reported.	The following MCC were determined: *Escherichia coli:* 10 ppm *Enterobacter aerogenes:* 20 ppm *Pseudomonas aeruginosa:* 20 ppm *Staphylococcus aureus*: 40 ppm All controls shown growth.	[redacted] 2007 (A5.2-01)
Inhibition of bacterial	PT 2,6, 11 and 12	Preventol P100: 99.0%	Bacteria: *Bacillus subtilis*,	Inhouse method No. 2304	Bronopol was added to	The following MIC were	[redacted] 2003 (A5.2-02)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
growth		bronopol	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Alcaligenes faecalis*, *Corynebacterium sp*. Mould fungi: *Penicillium brevicaule*, *Chaetomium globosum*, *Aspergillus niger*, *Rhodotorula rubra*, *Fusarium solani* and *Geotrichum candidum*	03001-02 98E and 2304-03002-02 98E from Bayer AG: Minimum Inhibitory Concentration (MIC) test	the nutrient substrate prior to the inoculation with microorganisms. Concentrations: Untreated samples, 3 ppm to 800 ppm Exposure: 3 days for *B. subtilis* and *P. aeruginosa*. 4days for *P. fluorescens*, *A. faecalis*, *Corynebacterium sp.*, *R. rubra*, *F. solani* and *G. candidum*. 14 days for *P. brevicaule*, *C. globosum* and *A. niger* The number of replicates is no reported.	determined: *B. subtilis:* 5 ppm *P. aeruginosa:* 10 ppm *P. fluorescens:* 5 ppm *A. faecalis:* 5 ppm *Corynebacterium sp:* 3 ppm *P. brevicaule:* 800 ppm *C. globosum*, *A. niger*, *R. rubra*, *F. solani* and *G. candidum*: >500 ppm All control shown growth.	
Inhibition of bacterial growth	PT 2,6, 11 and 12	Bronopol	Bacteria (gram-positive): *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium pyogenes, Bacillus subtillis* Bacteria (57ran-negative): *Pseudomonas aeruginosa, Proteus vulgaris, Proteus rettgeri, Proteus morganii, Proteus inconstans,*	Minimum Inhibitory Concentration (MIC) test	Bronopol was serially diluted in agar and surface inoculated. The inoculum was of 0.01 mL of 18 hrs broth cultures of the test bacteria or yeasts, or 0.01 mL of spore suspensions prepared from 7-day cultures of the fungi. After incubation at 37°C (bacteria) and at 26°C (yeast and fungi) the MIC was determined.	The following MIC were determined: Gram-positive bacteria: 12.5-50 µg/m (ppm) Gram-negative bacteria: 12.5-50 µg/mL Fungi: 100-400 µg/mL	[redacted] 1964 (A5.1-01)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			Proteus mirabilis, *Escherichia coli,* *Enterobacter aerogenes,* *Sallmonella typhosa,* *Salmonella typhimurium,* *Salmonella gallinarum,* *Salmonella enteritidis,* *Salmonella ser. Dublin,* *Salmonella ser. Heidelberg,* *Shingella sonei,* *Klebsiella pneumoniae* Fungi: *Trichophyton metagrophytes* *Trichophyton rubrum* *Trichophyton tonsurans* *Microsporum canis* *Clasosporium herbarum* *Penicillium roqueforti* Yeast: *Candida albicans*		Concentrations: 12.5 – 25 – 50 µg/mL (bacteria), 100 – 200 – 400 µg/mL (fungi and yeasts) No controls are reported. Exposure: 24 hrs (bacteria), 48 hrs (yeasts), 120 hrs (fungi) The number of replicates is no reported.	Yeasts: ≥400 µg/mL	
Inhibition of bacterial growth	PT 2,6, 11 and 12	Bronopol	Bacteria (gram-positive): *Staphylococcus aureus* Bacteria (gram-negative): *Pseudomonas aeruginosa,* *Proteus spp.,* *Escherichia coli,* *Salmonella spp.,* *Shingella spp.*	Minimum Inhibitory Concentration (MIC) test of Bronopol and other antimicrobial agents.	Bronopol was serially diluted in agar and surface inoculated. The inoculum was of 0.01 mL of 18 hrs cultures of the test bacteria, diluted 1/100 in water (except S. aureus). After incubation at 37°C the MIC was determined. Concentrations: 6.25 – 12.5 – 25 µg/mL	The following MIC were determined: Gram-positive bacteria: 12.5-25 µg/mL Gram-negative bacteria: 6.25-12.5 µg/mL	[redacted] 1978 (A5.1-02)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					No controls are reported. Exposure time: 24 hrs The number of replicates is no reported.		
Inhibition of bacterial growth and bactericidal	PT 2,6, 11 and 12	Bronopol	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa*	**1)** Minimum Inhibitory Concentration (MIC) test **2)** Bactericidal effect for *E. coli*.	**1)** Graded concentrations of bronopol and of other nitro compounds were prepared in 10 ml of nutrient broth and inoculated with suspensions of either *E. coli, S. aureus or P. aeruginosa* to give a final concentration of 10^6 cells/ml. An approximate bactericidal endpoint was determined by subculturing a loopful of medium from tubes showing no visible growth into a further 10 ml of medium. The procedure was used for cell concentrations of 10^2, 10^3, 10^4 and 10^5/ml. **2)** The bactericidal effect was determined for *E. coli* as a mean single survivor time (MSST) by the method of Mather	**1)** The following MIC were determined: *Escherichia coli*: 32 µg/mL *Staphylococcus aureus*: 35 µg/mL *Pseudomonas aeruginosa*: 15 µg/mL The approximate bactericidal endpoint for Bronopol was 10-20 µg/mL greater than the MIC value. All controls shown growth. **2)** The MSST for *E.coli* was: 108 min for 700µg/mL; 3-5 h for 1000µg/mL;	[redacted] 1973 (A5.4-01)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					from extinction data obtained by the technique of Berry & Bean using 25 replicates and 5 ml of neutralizing medium.	5 h for 2000µg/mL and 28 h for 5000µg/mL From this it seems that Bronopol is primarily a bacteriostatic agent against nonproliferating organisms.	
Inhibition of bacterial growth and bactericidal	PT 2, 6, 11, 12	Bronopol	*Escherichia coli*	**1)** Growth inhibition test.	**1)** Cultures were grown in a simple salt, chemically defined medium. When they were in the logarithmic phase of growth and E470 nm (optical density measurements) was 0.15, various concentratios of Bronopol were added. Concentrations applied: unpreserved, 4 to 20 µg/mL In selected experiments, cysteine was added at various times following the addition of biocide (in a concentration of one-half the MIC, so that both activation and neutralization of the growth inhibitory effects might be observed) to determine the ability of	**1)** After the addition of biocide to actively growing cultures o E. coli, growth inmediately ceased. Bronopol-induced bacteriostasis persisted for up to 90 minutes. When growth was resumed, it was at a lower rate than that of the control. The length of the bronopol-induced bacteriostasis was proportional to the applied concentrations. Estimated MIC:	[redacted] 1988 (A5.4-02)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					thiols to neutralize and reverse the growth inhibitory action of Bronopol. In other experiments, catalase (50 U/ml) or superosxide dismutase (60 U/ml) was added simultaneously with Bronopol. Exposure time: 2 hours 2 replicates	13 µg/mL. When the molar ratio of cysteine to Bronopol was 1:1, cysteine failed to alter the pattern of inhibition. However, at a 10:1 molar ratio the length of the induced bacteriostatic period was substantially reduced provided that the addition of cysteine was made less than 40 minutes after that of the biocide. In no case was the inhibited growth rate following the shortened bacteriostatic period increased by the presence of a neutraliser. The addition of catalase or superoxide dismutase caused no change in the	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
						pattern of inhibition.	
				2) Bactericidal test.	**2)** Washed suspensions of *E. coli* were equilibrated at 35ºC prior to the addition of biocide. At appropriate times, 1 ml portions were diluted in thioglycolate medium. Suitable dilutions (0.1 ml) were spread on the surfaces of predried nutrient agar plates. Experiments were repeated at various temperatures at pH 7.0 and at various pH values at 35oC. Selected experiments were performed in the presence of catalase (100 U/ml) or superoxide dismutase (200 U/ml) or under anoxic conditions. <u>Concentrations applied</u>: unpreserved, 100 – 200 – 300 – 400 - 500 µg/mL Exposure time: 16 hours 3 replicates	**2)** Bactericidal activity was approximated to first-order kinetics for concentratrions of bronopol greater than 100 µg/mL. Time survival data were redetermined for bronopol at 500 µg/mL under anoxic conditions and under aerobic conditions in the presence of catalase and superoxide dismutase. All three sets of conditions significantly reduced the degree of bactericidal activity. Such effects were particularly marked under anoxia and in the presence of superoxide	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
						dismutase. All controls shown growth.	
Inhibition of bacterial growth	PT 2, 6, 11, 12	Bronopol	Micro-organisms used are representative of those to be controlled in product types 2, 6, 11 and 12 are: *Bacillus cereus,* *Bacillus subtilis,* *Micrococcus flavus,* *Staphylococcus aureus,* *Staphylococcus epidermis,* *Streptococcus faecalis,* *Escherichia coli,* *Klebsiella aerogenes,* *Legionella pneumophila,* *Legionella micdadei,* *Legionella longbeachae,* *Legionella bozemanii,* *Proteus morganii,* *Proteus vulgaris,* *Pseudomonas aeruginosa,* *Pseudomonas cepacia,* *Pseudomonas fluorescens,* *Pseudomonas putida,* *Pseudomonas stutzeri,* *Salmonella typhimurium,* *Desulphovibrio desulphuricans,* *Desulphovibrio vulgaris,* *Candida albicans,* *Saccharomyces cerevisiae,*	In-house method to assess the Minimum Inhibitory Concentration (MIC)	Bronopol was incorporated into selected media at varying concentrations. The product was diluted in sterile distilled water in arithmetic dilutions (1:2 dilutions). 1 mL of each dilution was incorporated into 19 mL of agar to give the final dilution (1:20). The plates were dried at room temperature and inoculated with the test organisms. Water control plates were used. 2 replicates (where the replicates varied for any organism, the highest value obtained was quoted).	Bronopol was shown to have a MIC of between 12.5 and 50ppm for Gram-negative and Gram-positive bacteria. For Sulphur reducing bacteria the MIC was 12.5ppm for the strains tested. Against yeasts and moulds the MIC was found to be higher and ranged from 400ppm against *Stachybotrys atra* IMI 82021 to 6400ppm against *Trichoderma viride* Bowater 1096. All controls shown growth.	[redacted] 2006 (A5.3.1-01)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			Aspergillus niger, Chaetomium globosum, Cladosporium herbarum, Margarinomyces fasiculatis, Penicillium funiculosum, Stachybotrys atra, Trichoderma viride				
Bactericidal	PT 2, 6, 11, 12	Protectol BN: ≥99% bronopol	*Escherichia coli*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	Modified EN 1040 test under clean conditions, conducted over extended time periods to show significant measurable values. Validity checks and inoculum counts were performed according to EN 1040 and EN 1276 method.	Concentrations: Unpreserved, 100, 200, 500, 1,000 and 5,000 ppm Bronopol Contact time: 15, 60 mins, 3 hrs, 18 hrs and 24 hrs The aerobic bacteria were plated onto Tryptone Soya Agar (TSA) and incubated aerobically at 30±2°C for at least 48 hours. The *Legionella pneumophila* were plated to Legionella BCYE Agar plates and incubated aerobically at 37±2°C for at least 7 days. One replicate.	200 ppm of Protectol BN was shown to give a significant log reduction (>4 log) within 24 hrs for all organisms tested. 100 ppm of Protectol BN demonstrated at least a 2 log reduction within 24 hours, except against *S.aureus*. Control sample showed growth.	[redacted] 2007 (A5.3.1-02)
Algicidal	PT 11, 12	Bronopol	*Scenedesmus obliquus, Chlorella emersonii var. Globosa, Euglena gracilis*	Cidal test: The methods employed were a combination of the OECD Algae Inhibition Test Guideline'	A range of concentrations of bronopol were introduced into cultures of three freshwater algae to determine the algicidal concentration of bronopol.	Bronopol was found to be algicidal against *Scenedesmus obliquus* between 10 ppm and 30	[redacted] 1996 (A5.3.1_03)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				and the EPA Algicidal Test Guideline	1% (v/v) Algal pre-cultures were prepared containing 1x10^4 cells per mL and incubated at 24°C in a light cabinet employing 16 hours daylight and 8 hours dark cycles. Bronopol was added at 0, 10, 30 and 100 ppm a.s., the test solutions incubated for a period of 7 and 14 days. Number of replicates: 3.	ppm (a.s.). However, against *Chlorella emersonii var. globosa* and *Euglena gracilis* it appears that greater than 100 ppm a.s Bronopol is required. Although at 100 ppm Bronopol did control *Chlorella emersonii var. globosa* for 1 week.	

A.2.2.2 Mode of action

Bronopol reacts with thiol-groups of amino acids and enzymes (e.g. cysteine). Bronopol catalytically oxidises thiol-groups to disulphide bonds with rapid consumption of oxygen. This could account for the inhibition of enzyme activity ([redacted] 1973-A5.4-01; [redacted] 1988-A5.4-02). Bronopol is not destroyed during the oxidation of thiol-groups. This could explain the residual activity. Oxidation requires the presence of two –SH groups close enough together to make possible the formation of a –S–S– bond. If the –SH (thiol) groups are too far apart or lie in close proximity to electronegative polar groups, oxidation will not occur or be hindered ([redacted] 1973; A5.4-01).

[redacted] (1988; A5.4-02) observed a second, slower reaction that did not require oxygen and that consumed or neutralised Bronopol within the cell. In the absence of air, Bronopol seems to act as an oxidizing agent. Reduction of growth rate following the induced bacteriostasis probably reflects irreversible damage to the cell, possibly through the generation of oxygen radicals (e.g. superoxide and peroxide; [redacted] 1988; A5.4-02). The generation of superoxide by the aerobic reaction of Bronopol with thiols was demonstrated by the reduction of cytochrome *c* by superoxide.

The results suggest a dual action of Bronopol, with catalytic oxidation of accessible thiols being responsible for the growth inhibition and generation of free radicals causing cell death.

From the mode of action of Bronopol, it is clear that damage to essential metabolic activity occurs reasonably rapidly, however cell death is not instantaneous, and it occurs more gradually over time. Bronopol finds use in applications where a sustained antimicrobial effect is required and initial inhibition of growth leading to eventual cell death is sufficient for use.

Bactericidal activity against *E. coli* was approximated to first-order kinetics for concentrations of Bronopol greater than 100 µg/mL ([redacted] 1988; A5.4-02). The activity increased with increasing pH from pH 5.5 to 8. Bronopol is stable in acidic conditions, but chemically less stable in alkaline systems. However, in-use experience has shown that Bronopol is an effective preservative in alkaline systems (see Anonymous 1992)[1]. At pH above about 8.5-9.0, Bronopol will not be long lasting as an in-can preservatives due to lack of chemical stability.

A.2.2.3 Resistance

The mode of action of Bronopol is complex and multi point, therefore the development of resistance is less likely than for those biocides that have a simple single target site of action. Bronopol-resistant organisms were not produced in the laboratory when four strains of *Pseudomonas aeruginosa* and three strains of *Staphylococcus aureus* were exposed daily for 20 days in the presence of sub-lethal concentrations of Bronopol (Anonymous 1992).

However, resistance mechanisms are theoretically possible and usage patterns need to be geared towards reducing the chances of this occurrence. To prevent development of resistance it is common to dose products or systems with more than one biocide at once or to alternate treatment regimes.

A.2.2.4 Conclusion on efficacy

Experimental data on the effectiveness of the active substance against target organisms are summarised in the Table A-16.

Bronopol was shown to be a highly effective antimicrobial agent when tested in standard biocide efficacy tests. Minimum Inhibitory Concentration (MIC) studies and suspension tests were conducted to demonstrate the lowest level of biocide which inhibits the growth of common spoilage microorganisms. The results showed that Bronopol was effective at concentrations ranging from 2 to 6400 parts per million (ppm) for the microorganisms tested. Bronopol was more effective versus bacteria, with range MICs of 2 to 400 ppm. The MIC values versus yeast were 200 to 400 ppm and values versus mould ranged from 100 to 6400 ppm. Basic efficacy against the algae *Scenedesmus obliquus* at 10-30 ppm was also observed.

[1] Anonymous 1992. Bronopol (BNPD) - Bronopol-Boots* BP, Myacide* BT - A broad spectrum antibacterial agent. Technical Bulletin, issue 6, February 1992. The Boots Company PLC, Nottingham, England.

A.3 Assessment of effects on Human Health

A.3.1 Toxicokinetics

Table A-17: Summary table of toxicokinetic studies

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
US EPA Guideline No. 85-1, *in vivo* **GLP** **Rel. 1** **Key**	Rat, Charles River CD, male and female, 5 animals/sex/group	TS: [2-^{14}C] Bronopol, specific activity: 65.9 MBq/g Dose: 10 mg/kg bw Route of administration: Oral, gavage Observation period: 168 h (7 days)	Extensive absorption in both, male and female rats. Main excretion (72-73%) via urine in both, male and female rats, with about 70% of the total urine excretion occurring during the first 24 h following dosage. Minor excretion was observed via faeces were faeces (10-11%), and expired air (≤ 4%). Distribution in organs/tissues: highest concentrations of the radioactive material were found in the liver (male: 0.236 µg/g; female: 0.108 µg/g) and lungs (male: 0.199 µg/g; female: 0.087 µg/g). Ratio of		[redacted] 1993 A6.02_01

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			the concentrations in whole blood to plasma was 3.4 and 2.3 in male and female rats, respectively.		
US EPA Guideline No. 85-1, *in vivo* **GLP** **Rel. 1** **Key**	Rat, Charles River CD, male and female, 6 males and 5 females/group	TS: [2-^{14}C] Bronopol, specific activity: 32.0 MBq/g Dose: 50 mg/kg bw Route of administration: Oral, gavage Observation period: 168 h (7 days)	2 of the 6 males suffered from respiratory distress and were sacrificed *in extremis*. Extensive absorption in both, male and female rats. Main excretion (68-79%) occurred via urine in both, male and female rats, with about 64 - 75% of the total urine excretion occurring during the first 24 h following dosage. Minor excretion was observed via faeces (12-14%) and expired air (≤ 7.5%). Distribution in organs/tissues: Highest concentration of the radioactive material was found in the lungs (male: 0.951 µg/g; female: 1.175 µg/g), fatty tissue		[redacted] 1993 A6.02_02

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			(male: 0.990 µg/g; female: 0.834 µg/g) and kidneys (male: 0.814 µg/g; female: 0.921 µg/g). Ratio of the concentrations in whole blood to plasma was 2.8 and 1.8 in male and female rats, respectively.		
US EPA Guideline No. 85-1, *in vivo* **GLP** **Rel. 1** **Key**	Rat, Charles River CD, male and female, 7 animals/sex/group were dosed, however, only 5 animals/sex/group were used for sampling and tissue distribution.	TS: [2-^{14}C] Bronopol, specific activity: 170.2 MBq/g Dose: Unlabelled Bronopol at a dose of 10 mg/kg bw for 14 consecutive days followed by a single dosage of 10 mg/kg bw ^{14}C-labelled Bronopol. Route of administration: Oral, gavage Observation period: 168 h (7 days)	Extensive absorption in both, male and female rats. More than 90% of the ^{14}C was absorbed and a half-life of approximately 3 hours was determined. Main excretion (67-76%), occurred via urine for both, male and female rats, with about 90% of the total urine excretion occurring during the first 12 h following the last dosage. Minor excretion was observed via faeces (*ca*. 3%) and expired air (*ca*. 9%). Distribution in organs/tissues: Highest concentration of the radioactive		[redacted] 1993 A6.02_03

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			material was found in the skin, kidneys and liver of male and female rats. Ratio of the concentrations in whole blood to plasma was 1.5 for both sexes.		
US EPA Guideline No. 85-1, *in vivo* **GLP** **Rel. 1** **Key**	Rat, Charles River CD, male and female	Urine samples from first the three studies mentioned above were examined for Bronopol metabolites.	Major Bronopol metabolite in urine of rats was found to be 2-nitropropane-1,3-diol. No Bronopol was found in the urine samples.		[redacted] 1993 A6.02_04
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** **Supportive**	Rat, CFY strain, male and female, 6 animals/sex for the metabolism study. 1 animal/sex for the biliary excretion study. 6 male rats from the metabolism study were used for autoradiography.	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1 mg/kg bw Route of administration: Oral, gavage Toxicokinetic was assessed by means of metabolism, biliary excretion and whole-body autoradiography.	Main excretion (83%) occurred via urine for both, male and female rats, with about 81% of the total urine excretion occurring during the first 24 h following the last dosage. Minor excretion was observed via faeces (6%) and expired air (8%). About 2% of the applied radioactivity was found in the carcass. Up to 7% of the applied radioactivity was found to be excreted with the bile within 48 h after application.		[redacted] 1974 A6.02_05a

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			Peak plasma ^{14}C concentration was reached 0.5-3 h after dosage with a $t_{1/2}$ of 3-5 h. The total radioactivity found in the plasma accounted for 3% of the administered dose. Five metabolites were found in the urine. One major metabolite accounting for >40% of the urine metabolites was identified as 2-nitropropane-1,3-diol. No Bronopol compound detected in the urine. Whole-body autoradiography showed rapid absorption (15 to 60 min) of ^{14}C-Bronopol and no persistence of Bronopol and/or its metabolites in the organs and tissues 24 h after application of the test substance.		
Non-guideline, *in vivo* Non-GLP Rel. 2	Beagle dog, male and female 4 male and 1 female	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1 mg/kg bw	Main excretion (81%) occurred via urine for both, male and female rats, with up to 64% of		[redacted] 1974 A6.02_05b

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
Supportive		Route of administration: Oral, gelatin capsules	the total urine excretion occurring during the first 12 h following the last dosage. Minor excretion was observed via faeces (3%). Peak plasma ^{14}C concentration was reached 0.5-2 h after dosage with a $t_{1/2}$ of about 4 h. The total radioactivity found in the plasma accounted for 6-9% of the administered dose. Five metabolites were found in the urine. One major metabolite accounting for >40% of the urine metabolites was identified as 2-nitropropane-1,3-diol. No Bronopol compound detected in the urine. Little variation was observed in the tissue distribution of ^{14}C in the organs. Highest concentration was found in the kidneys and lowest in fatty		

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			tissue.		
Non-guideline, *in vivo* Non-GLP Rel. 3 Supportive	Rat, CFY, 6 animals	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1.2 mg/kg bw (approx. 0.24 mg/cm^2 in the main study) Vehicle: Acetone Route of administration: Dermal, occlusive Exposure time: 5 days	Percutaneous absorption of Bronopol through the skin of rats was considered low since 79 – 107% of the applied radioactivity remained on the application site and the dressing. Main excretion (11% of the applied ^{14}C) occurred via urine within 48 h after application of the substance. Minor excretion was observed via faeces (≤ 1.3%) and expired air (≤ 6.5%). Only small amounts (2-3%) of the total ^{14}C applied remained in the carcass. Five Bronopol metabolites but no Bronopol were found in the urine, the major metabolite was identified to be 2-nitropronane-1,3-diol.		[redacted] 1974 A6.02_05c
Non-guideline, *in vivo* Non-GLP	Rabbit, New Zealand White 4 animals	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg	Up to 75% of the applied ^{14}C remained on the skin of the		[redacted] 1974 A6.02_05d

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
Rel. 2 **Supportive**		Dose: 0.72 mg/kg bw (approx. 0.096 to 1.192 mg/cm^2) Vehicle: Acetone Route of administration: Dermal, occlusive Exposure time: 4 days	application site (superficial penetration being restricted to the areas surrounding hair follicles) and the dressing. Five Bronopol metabolites but no Bronopol was found in the urine, the major metabolite was identified to be 2-nitropronane-1,3-diol.		
US EPA Guideline No. 85-1, *in vivo* **GLP** **Rel. 1** **Supportive**	Mouse, CFLP, male 4 animals/group	TS: [2-^{14}C] Bronopol, specific activity: 15.8 µCi/mg Dose: 1.5 mg/animal (approx. 0.67 mg/cm^2) Vehicle: Acetone/water 9:1 Route of administration: Dermal, covered Exposure time 1) Single application for up to 48 h. 2) Second application 48 h after the first one	Administered ^{14}C was released slowly into the plasma. After each application, approximately 5% of the applied ^{14}C remained in the skin. After the second dosage levels increased in skin but dropped in plasma. Metabolic profiles were similar in skin and plasma. One single metabolite 2-nitropropane-1,3-diol was identified in the majority of the samples. No parent compound was		[redacted] 1993 A6.02_06

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			observed in skin and plasma. Assuming a body weight of 20 g per mouse, the applied dose accounts for about 75 mg/kg bw.		
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** **Supportive**	Mouse, CFLP, male 4 animals/group	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1.5 mg/animal (based on a mean bw of 25 g, approx. 60 mg/kg bw) Vehicle: Acetone/water 9:1 Route of administration: dermal, w/o occlusion Exposure time: 30 days, 13 applications, 3 days/week	^{14}C was absorbed via skin and rapidly cleared from plasma with $t_{1/2}$ of *ca.* 8 h, accumulation of test substance was not observed. Plasma profiles of metabolites were similar on day 1 and day 29. Maximum plasma levels of ^{14}C were not affected by an interval of 48 or 72 h between consecutive dosing.		[redacted] 1987 A6.02_07
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** **Supportive**	Rat, Charles River CD, male 4 animals/group	TS: Bronopol, purity ≥98% Doses: 1 and 50 mg/kg bw Route of administration: Oral, gavage. Urine of Bronopol treated rats was	1 mg/kg bw: Bromide concentration in the urine was not above the endogenous level. 50 mg/kg bw: Within 120 h after application of the substance about 15-20% (w/w) of the applied Bronopol dose as excreted as	The capillary electrophoresis method used was especially developed for the purpose of bromide ion detection in rat urine ([redacted] 1993-A6.02_08_b)	[redacted] 1993 A6.02_08_a [redacted] 1993 A6.02_08_b

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
		examined for presence of bromide ion as a breakdown product of Bronopol.	bromide.		
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** **Supportive**	Rat, Schoe:WIST Male and female, 5 per sex (single and 5x application) 4 per sex (7x application)	TS: ^{14}C Bronopol (no further details reported) Vehicle: water Doses: 25 mg/kg bw (5 mg/rat corresponding to 430 kBq) for single and multiple application (5x) 20 mg/kg bw/day for multiple application (7x) Route of administration: Oral gavage Depending on the experiment urine, faeces, exhaled air, organs and/or blood were sampled.	After single application: Bronopol was rapidly absorbed from the gastrointestinal tract, T_{Cmax} and $T_{Cmax/2}$ in blood were at 1-2 h and 7.5-14 h, excretion was also rapid with about 86% of applied dose excreted within 24 h. Excretion was primarily via urine (73-75%) with a considerable amount also via expired air (CO_2, 8%) and via faeces (6-9%). Total bioavailability was at least 82-83% of applied dose. Total residues (carcass) were about 7-10% at 24 h after single application and highest tissue residues were seen in muscle, blood, and liver. In agreement with the observed rapid		[redacted] 1987 A6_02-1

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			elimination, multiple applications did not reveal a relevant accumulation potential		
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** **Supportive**	Rat and dogs, CFY (rat) and dog strain not reported Male and female, Mass balance – 6 rats, 2 dogs, 1 rat/sex for bile cannulation and 2 male dogs for tissue kinetic	TS: 2-^{14}C Bronopol, (specific activity: 21 µCi/mg, radiochemical purity: ≥99%) Vehicle: aqueous solution Doses: Single application, Rats: 1 mg/kg bw (corresponding to 5 µCi) Dogs: *ca*. 1 mg/kg bw (2 mg radiolabelled Bronopol + 6-8 mg unlabelled Bronopol) Route of administration: Oral, gavage (rats), capsule (dogs) Depending on the species urine, faeces, expired air, carcass, cage washing, tissues and/or blood were sampled. In addition, metabolite pattern was determined in urine and plasma.	Absorption, elimination: Blood kinetics revealed rapid absorption of Bronopol from the gastrointestinal tract and peak plasma concentrations were seen after 1-1.5 h with an initial half-life time of about 5 h in both species. Within 24 h after application 87% were excreted in rats (including expired air) and 77% in dogs (expired air not determined). Excretion was predominantly via urine (83.3% and 81.1% of applied dose in rats and dogs within 120 h after application), with a substantial part via expired air (8.4% in rats) and only a minor part via faeces (5.8% and 3.1% in rats and		[redacted] 1976 A6_02-2

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			dogs). Total excretion was 98% and 85% of applied dose for rats (including expired air) and dogs, respectively. Total bioavailability was 93% (including expired air) and 82% for rats and dogs. Distribution: Rapid and even distribution into tissues was seen in the rat without a potential for accumulation, no relevant tissue residues were detected in rats 24 h after application or at later time points. Radioactivity was also evenly distributed in dogs sacrificed at 1.5 h (T_{Cmax} in plasma) and 6 h ($T_{Cmax/2}$) after application, with exception of the kidney (residues exceeding plasma levels) and fat (lowest residue concentration). In parallel to the plasma level total tissue residues at 6 h (19.2% of applied dose) had decreased to half the		

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			values at 1.5 h (41.2%). Metabolism: Metabolite pattern in urine were similar in rats and dogs; five metabolite fractions were found. Bronopol was not detected, and it could be shown that the formation of cysteine conjugates was unlikely. NPD was also detected in plasma of rats and dogs. The amounts decreased from about 60% of plasma radioactivity at 0.25 h after application to 40% after 4 h, 30% after 5 h and 12% after 24 h.		
OECD 417, *in vivo* **GLP** **Rel. 2** **Key**	Rat, Crl:CD(SD)-Sprague Dawley derived Male 4 animals (groups 1-5) 2 animals (controls)	TS: Bronopol (purity: 99.85%) or ^{14}C Bronopol (specific activity 24.58 mCi/mmol, radiochemical purity 96.6%) Vehicle: acidified water (pH 4) Dose: Single application:	Bronopol was rapidly absorbed from the gastrointestinal tract and also rapidly eliminated predominantly via urine (69-75% of dose) and to minor amounts via expired air (3.1-3.9% of dose) and faeces (10-17% of dose) essentially		[redacted] 2007 A6_02-5

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
		group 1, 3, 4: 1 mg/kg bw, corresponding to 0.2 mg/g dosing solution group 2: 30 mg/kg bw, corresponding to 6.0 mg/g dosing solution group 5: Multiple application: 14 x 1 mg/kg bw/day (unlabelled) followed by 1 mg/kg bw (labelled) <u>Route of administration:</u> Oral gavage Depending on the group urine, faeces, expired CO_2, organ/tissues (blood cells, plasma, fat, gastrointestinal tract, kidneys, liver, skin, spleen, residual carcass), cage wash and/or blood were collected and analysed at different time points.	independent of dose level and pre-treatment. At the high dose level maximum blood levels were seen within 1 h after application and eliminated with an initial half-life time of 3-4 h. Distribution to and elimination from tissues was as rapid as from blood. Highest residue levels were seen in liver and kidney, lowest values in fat. Comparison of tissue residues after application of a single low or high dose level or multiple applications of the low dose, did not reveal a relevant potential for accumulation except marginally higher residues in liver and kidney after multiple application.		
Non-guideline, *in vivo* Non-GLP	Rat and rabbit, CFY (rat), New Zealand White (rabbit),	<u>TS:</u> 2-^{14}C Bronopol (specific activity: 21 µCi/mg radiochemical	Total dermal absorption including potential skin residues		[redacted] 1976 A6_02-3

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
Rel. 3 Supportive	Sex and No. of animals not reported	purity: ≥99%) Vehicle: water, acetone, acetone:water = 9:1 v/v (rat); acetone (rabbit) Dose: 1 mg/kg bw corresponding to 0.25 mL/kg bw, assuming a bw of 200 g for rats and 3 kg for rabbits, this corresponds to 50 µL (rat) and 0.75 mL (rabbit) Route of exposure: Dermal Duration of exposure: 6-48 h (rabbits), 6-120 h (rats)	in the rat may therefore be estimated as follows: in absence of organic solvents: 4% from excretion within 24 h (vehicle water) plus 3% from carcass (value from up to 3 days exposure with vehicle acetone) plus up to 20% skin residues after washing (value from experiment with vehicle acetone), resulting in about 27% of applied dose. When acetone is used as vehicle the respective calculation results in 30% of applied dose. Assuming similar skin residues after washing as shown in the experiments with rats, the total dermal absorption after 24 h of exposure may be about 31% of applied dose (vehicle acetone) in rabbits, similar to the rat.		
Non-guideline, *in vivo* Non-GLP	Rat, Wistar Female	TS: 2-^{14}C Bronopol (specific activity: 15.4	After intravenous application about 85%		[redacted] 1980 A6_02-4

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
Rel. 2 **Key**	Dermal: 7 Intravenous: 6	µCi/mg, radiochemical purity: 99%) <u>Vehicle:</u> Dermal: acetone Intravenous: 0.9% NaCl solution <u>Dose:</u> Dermal: 1 mL/kg bw corresponding to 10 mg/kg bw (considering the range of body weights and the size of the test site this is equivalent to 42-45 µL/cm^2 corresponding to 0.42-0.45 mg/cm^2) Intravenous: 2 mL/kg bw (10 mg/kg bw) <u>Route of exposure:</u> Dermal or intravenous	of the applied dose were excreted within 24 h. The major part in urine (74%) with a considerable amount in expired air (9%) and a small part in faeces indicating biliary excretion (1.3%). After dermal application about 19% of the applied dose was excreted corresponding to 22% of the amount excreted after intravenous application. Considering also carcass residues a dermal absorption of about 34% of applied dose may be assumed. As skin wash and skin stripping were not performed, it is difficult to evaluate whether the amount remaining to be associated with the unwashed treated skin (59.5%) should be considered as systemically available or not. The study authors		

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
			reported the dermal penetration to be 40% corresponding to the amount of compound not being associated with the treated skin.		
Absorption and excretion of ^{14}C-labelled Bronopol after dermal application to volunteers **Non-guideline** **Non-GLP** **Rel. 3** **Supportive**	Human 2 volunteers	Test Substance: ^{14}C-labelled Bronopol (specific activity: 1.4 µCi/mg; radiochemical purity: ca. 97%) Vehicle: Soltan cream (pharmaceutical formulation) The absorption and excretion of ^{14}C-labelled Bronopol was examined in human following application of a cream containing 0.1% of the test material (Soltan 3 cream) to the skin.	The maximum amount of ^{14}C-labelled Bronopol, which could have been available systematically to the volunteers was about 34% for volunteer A and 8% for volunteer B	Low findings of radioactivity in urine and faeces indicate low systemic availability of Bronopol due to little absorption. No precise quantitative conclusions can be made due to the variation seen between the volunteers and the small number of volunteers.	[redacted] 1984 B6.7_01_a
Investigation of the absorption Bronopol in volunteers following the application to the skin of Soltan 3 cream containing 0.1% Bronopol **Non-guideline** **Non-GLP**	Human 10 volunteers, 5 males and 5 females	Test Substance: Bronopol Vehicle: Soltan cream (pharmaceutical formulation) All items used for application and removal of the test	After 8 h, 52 to 87% (mean: 72%) of the applied Bronopol was recovered after washing of the application site. Recovery in females ranged between 71% and 87% (mean: 80%)		[redacted] 1984 B6.7_01_b

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results	Remarks (e.g. major deviations)	Reference
Rel. 3 Supportive		material from the skin of the treated volunteers were collected and the cream adhering to them was washed off into distilled water. The cream was broken down by agitation (mechanical or magnetic stirrer) to allow dissolution of the Bronopol. The combined washings from each volunteer were diluted to a standard volume of 500 ml for analysis. Four ml aliquots were prepared and subjected to high performance liquid chromatography (HPLC); the assay method was sufficiently sensitive for detection of Bronopol at the concentration levels used in the present study.	whereas in males, recovery ranged between 57% and 67%. Mean Bronopol recovery in female volunteers following single application of 0.1% Bronopol in Soltan cream on the abdominal skin was about 80% whereas in male volunteers a mean recovery of 63% was reported. The author suggested that the difference between males and females might be related to the increased incidence of hair follicles in the male abdominal wall and/or the coarse hair covering the male abdominal wall.		

A.3.1.1 Short summary and overall relevance of the provided toxicokinetic information

A.3.1.1.1 Oral route

The toxicokinetics of bronopol after single and repeated oral application of [2-^{14}C]-bronopol to male and female CD rats was assessed in GLP conform studies following Guideline 85-1 of the Office of Pesticide Programs (OPP), US-EPA, which is similar to the OECD test guideline 417 ([redacted] 1993-A6.02_01, [redacted] 1993-A6.02_02, [redacted] 1993-A6.02_03). Absorption of the applied ^{14}C in male and female rats after a single oral dose of 10 and 50 mg/kg bw was reported to range from 75 to 100% with no differences between male and female animals. Independently from the gender and the dose applied, the main excretion occurred via the urine where 68-79% of the applied radioactivity was recovered during an observation period of 7 days. Furthermore, 64-75% of the total radioactivity recovered in the urine was excreted during the first 24 h. Minor excretion of the orally applied radioactivity occurred via the faeces (10-14%) and the expired air (<8%), where it was measured as $^{14}CO_2$. Distribution of the orally applied ^{14}C in organs and tissues was examined 7 days after the administration. For male and female rats of both dose groups, the average radioactivity remaining in organs and tissues at the end of the observation period was within the range of 0.5-1.0% of the applied radioactivity. For the 10 mg/kg bw group, most radioactivity was found in the liver and lungs of male and female rats, whereas in the 50 mg/kg bw group, lungs, fatty tissue and kidneys in rats of both gender were reported to show the highest level of radioactivity. Independently of the bronopol dose applied, the average ratio of the ^{14}C concentration in whole blood to plasma was in general slightly higher in male than in female rats at the end of the observation period, i.e. 2.8-3.4 and 1.8-2.3, respectively ([redacted] 1993-A6.02_01, [redacted] 1993-A6.02_02).

Toxicokinetics observed after repeated oral application of Bronopol ([redacted] 1993-A6.02_03) showed the same characteristics than the kinetics after a single oral application. Charles River CD rats of both gender were dosed orally with 10 mg/kg bw of unlabelled bronopol for 14 consecutive days, followed by a single [2-^{14}C]-bronopol dose of 10 mg/kg bw. Excretion and distribution of the applied radioactivity was monitored for 7 days after the last application. More than 90% of the applied ^{14}C was considered to be absorbed in both male and female rats. Main excretion occurred via the urine where 67-76% of the applied radioactivity was recovered during the observation period of 7 days. About 90% of the total radioactivity recovered in the urine was excreted during the first 12 h. Minor excretion of the orally applied radioactivity occurred via the faeces (*ca*. 3%) and the expired air (*ca*. 9%), where it was measured as $^{14}CO_2$. As a result a half-life time of *ca*. 3 h was determined for bronopol when applied repeatedly by gavage. At the end of the observation period a total of 3% of the applied ^{14}C was found in organs and tissues of male and female rats with the highest concentrations in skin, kidneys and liver. The average ratio of the ^{14}C-concentration in whole blood to plasma at the end of the observation period did not differ between male and female and was reported to be 1.4 and 1.5, respectively ([redacted] 1993-A6.02_03).

Doses of 1, 20 and 25 mg/kg bw ([redacted] 1987-A6_02-1; [redacted] 1976-A6_02-2) were tested in rats and dogs. After single oral application, peak levels in plasma of bronopol were reached within 1-2 h, meaning a rapid absorption both at 1 mg/kg bw in rats and dogs and at 20 and 25 mg/kg bw in rats.

Initial plasma half-life times were about 5 and 6.5-12 h at 1 and 20 and 25 mg/kg bw, respectively. The radioactivity was rapidly excreted independent of sex and dose level predominantly via urine (69-83% of applied dose within 24 h). Total excretion was 88-98% within 72-120 h after application. Amounts of 3-8% of applied dose were detected in expired air (CO_2). Excretion via faeces played only a minor role (6-17% of applied dose).

No relevant species difference was seen between rats and dogs. Similar to the rat total excretion was about 85% of applied dose after single oral application of 1 mg/kg bw in dogs ([redacted] 1976-A6_02-2). Excretion was primarily via urine (81% of applied dose) with a minor amount via faeces (3%). Expired air was not determined in dogs but can be expected to be similar to the rat.

In plasma T_{Cmax} was 1.5 h and the half-life time about 5 h in dogs. Tissue residues followed blood kinetics (values at 6 h after application about half of the values at 1.5 h after application). Except

for the kidney, tissue concentrations were lower than blood. 2-nitropronane-1,3-diol (NPD) was identified as the main metabolite corresponding to 44% of applied dose. No bronopol was detected in urine.

In a guideline study ([redacted] 2007-A6_02-5) 5 male rats were given by gavage a single application of 1 mg/kg bw, a single application of 30 mg/kg bw or a multiple application of 14 x 1 mg/kg bw. The bioavailability was considered to be higher than 80% for all doses levels. The pattern of distribution and elimination did no reveal any potential for accumulation.

A.3.1.1.2 Dermal route

Toxicokinetics of bronopol in rats and rabbits following dermal application were reported in two non-GLP, non-guideline studies ([redacted] 1974-A6.02_05c; [redacted] 1974-A6.02_05d). For both studies acetone had been chosen as a vehicle since a pre-test showed highest dermal penetration of the test substance when dissolved in acetone than for acetone/water (9:1) or water alone. Furthermore, no difference in skin penetration was observed when comparing the acetone/water mixture and water alone.

A single dose of 1.2 mg/kg bw [2-^{14}C]-bronopol was applied to an area of 1 cm^2 on the hairless back of 6 CFY rats under occlusive conditions for four days ([redacted] 1974-A6.02_05c). Though the vehicle allowing maximum dermal penetration was chosen, about 11% of the substance was considered to be absorbed percutaneously but most of the applied radioactivity, namely 77-106% remained on the application site. It is not specified if this percentage of the radioactividty remained in the application site after the washing of the area or was already absorbed by the skin. About 11% of the applied radioactivity was excreted via the urine, mainly during the first 48 h following application. Minor excretion was observed via faeces (≤ 1.3%) and expired air (≤ 6.5%) and about 2-3% of the total ^{14}C applied remained in the carcass at the end of the observation period. 5 bronopol metabolites but no bronopol were found in the urine. The major metabolite was identified to be NPD.

[2-^{14}C]-Bronopol applied dermally at a dose of 0.72 mg/kg bw to an area of about 10 cm^2 on the clipped backs of four New Zealand White rabbits under occlusive conditions for four days remained for the most part (43-75% of the applied radioactivity) on the skin and the dressing ([redacted] 1974-A6.02_05d). It is not specified if a washing was performed to remove the applied substance from the skin. Microautoradiography of skin samples showed the presence of radioactivity within the epidermis, with superficial penetration in the areas surrounding hair follicles. No radioactivity could be evidenced in the dermis, muscle layers or in untreated skin areas. Main excretion (up to 25% of the applied ^{14}C) occurred via urine within 48 h after application of the substance. Minor excretion was observed via faeces (< 1%). Most ^{14}C within organs and tissues was found in muscles (1-2%) and fatty tissue (0.5-3%). 5 bronopol metabolites but no bronopol were found in the urine, the main metabolite was identified to be NPD.

The toxicokinetics of [2-^{14}C]-bronopol after single and repeated dermal application to male mice was assessed in a GLP conform study following guideline 85-1 of the Office of Pesticide Programs (OPP), US-EPA ([redacted] 1993-A6.02_06). Single dosed mice received a single application of [2-^{14}C]-bronopol for up to 48 h. Repeatedly dosed mice received their second and last treatment 48 h after the first application for an additional 24 h. The applied doses for single and repeated dose were 75 mg/kg bw. After a single dermal application of bronopol 11-15% of the applied radioactivity remained on the skin or in the dressings, whereas 8-11% of the applied radioactivity remained unabsorbed after repeated application. For both single and repeated dosage the plasma concentration of ^{14}C peaked 0.5-1 hour after application and decreased by approximately 60% over the following 24 h. 5-6% of the applied radioactivity was found in the skin 0.5 h after single and 1 h after repeated application. After single exposure radioactivity found in skin decreased by 40% during the first 24 h, however, it was found to remain constant for the next 24 h, whereas the data for repeated exposure were less conclusive. No unchanged bronopol was found in the plasma and skin of the treated animals but one and occasionally a second metabolite. The main metabolite was identified as NPD whereas the second metabolite remained unknown. After single application 70-88% of the radioactivity found in the plasma during the first 24 h were attributed to the main metabolite NPD, whereas for the twice

treated animals a decrease in plasma levels of NPD by 80% during 24 h was observed. In this part of the study a second, however unknown metabolite was detected. In the skin, 80-98% of the detected levels of radioactivity were attributed to presence of NPD, which did not depend on the dosage and did not change significantly during the observation period.

A dermal absorption study ([redacted] 1976-A6_02-3) where rats and rabbits were exposed to 1 mg/kg bw of bronopol was performed. Dermal absorption of bronopol in rats after 24 h was 4% in water and 7% in acetone (penetration enhancer). Absorption after 120 h was estimated to be 10 and 20% of the applied dose in rats with water and acetone as vehicle, respectively. In rabbit, the absorption of bronopol in acetone was 11% in 24 h, and 35.6% in 48 h.
Effectiveness of washing was tested separately and it was shown that the majority of the applied dose could be removed from the skin.

For human risk assessment, the dermal penetration was calculated from the metabolism study with topical administration ([redacted] 1980-A6_02-4) because other dermal studies present several inconsistencies and the value obtained from this study acts as a worst case. In this study, according to the EFSA Guidance on dermal absorption, since the recovery was < 95%, the dose not detected in the skin has been considered as absorbed, that is 42.9% estimated from the amount of radioactivity detected in excreta, cage washings, tissues and carcass. Although 7 animals were used in the study, only in 2 of them the carcass was analysed, so the corresponding k = 1.6 was applied. The standard deviation has been calculated from the deviation of the radioactivity measurements in each compartment, so s = 4.5. In conclusion, the percutaneous absorption was estimated at 50.1%.

A.3.1.1.3 Metabolites

The urine samples obtained from the toxicokinetics studies reported in [redacted] 1993-A6.02_01 to [redacted] 1993-A6.02_03 had been used of identification of bronopol metabolites ([redacted] 1993-A6.02_04). Urine samples collected during the first 24 h after application of [2-^{14}C]-bronopol were pooled within the same sex and group and analyzed by high pressure liquid chromatography (HPLC), liquid chromatography - mass spectrometry (LC-MS) and thin layer chromatography (TLC). The main metabolite in the urine found after HPLC and TLC analysis was identified to be NPD. HPLC profiles of the urine of male and female rats of any treatment group did not differ significantly between genders, except for the females of the 50 mg/kg bw single dose treatment group, where the concentration of the main metabolite was the lowest. Still, the mean relative amount of the main metabolite NPD found in the urine of bronopol treated male and female rats accounted for approximately 50% of the total radioactivity as confirmed by both HPLC and TLC analysis. Though 1 to 3 additional metabolites were seen on the chromatograms their molecular structure could not be resolved. In none of the treatment groups any unchanged bronopol could be detected in the urine of the male and female animals.

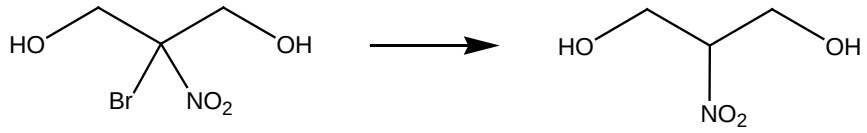
Toxicokinetics of bronopol in rats discussed so far is consistent with findings reported in a valid non-GLP, non-guideline study in which male and female CFY rats received a single oral dose of [2-^{14}C]-bronopol of 1 mg/kg bw ([redacted] 1974-A6.02_05a). In addition to rats, toxicokinetics of bronopol has been examined in 3 male and 1 female Beagle dogs, receiving a single oral dose of 1 mg/kg bw in a gelatine capsule ([redacted] 1974-A6.02_05b). Urine and faeces were collected daily from 2 animals (1 male and 1 female) during 5 days. Following single oral dosage, at least 84% of the applied bronopol was absorbed in male and female Beagle dogs. The main excretion occurred via the urine where more than 80% of the applied radioactivity was recovered during the observation period of 5 days. Furthermore, 64% of the total radioactivity recovered in the urine was excreted during the first 12 hours. Minor excretion of the orally applied radioactivity occurred via the faeces (3%). The main metabolite in the urine of the Beagle dogs accounting more than 40% of the applied dose was identified to be NPD whereas no unchanged bronopol was found. Peak plasma concentration of radioactivity was reached within 30 min to 2 h, and the half-life of bronopol and metabolites in plasma was *ca*. 4 h. The tissue distribution showed that the highest concentration of ^{14}C was found in the kidneys and lowest in fatty tissue. Thus, the toxicokinetic of orally applied bronopol in dogs is in good accordance with the toxicokinetics observed for rats.

Blood samples of CFLP mice were analysed for bronopol and its metabolites during repeated dermal

application of [2-^{14}C] bronopol at a dose of 60 mg/kg bw ([redacted] 1987-A6.02_07) for a total of 13 times during 30 days (3 days/week). Since the application site was not occluded the following results may reflect the toxicokinetic of a mixed oral and dermal exposure to bronopol. About 1 hour after dosing ^{14}C concentration peaked in the plasma but declined rapidly leading to graphically-determined plasma half live time $t_{1/2}$ of 8 h. After TLC analysis the fingerprints of the metabolites in the plasma were similar on day 1 and day 29. A total of 5 spots were observed on the chromatograms 1 of which co-eluted with a bronopol standard. However, since no other known compounds were tested in co-chromatography it remains unclear whether it was indeed bronopol which was found in the plasma.

Detection of Br^- in urine was investigated in male CD rats given single oral doses of 1 and 50 mg/kg bw of bronopol ([redacted] 1993-A6.02_08_a). That detection was based on a capillary electrophoresis method, which had been especially developed for this purpose ([redacted] 1993-A6.02_08_b). Following the single oral dose of 1 mg/kg bw, Br^- was not detectable above the endogenous level in the urine of treated animals. In the 50 mg/kg bw group, maximum excretion rate of Br^- (15-25 µg/h) in the urine was observed between 8 and 24 h post-dosing. Excretion of bromide in the urine during 120 h in the 50 mg/kg bw group was calculated to account for 17% (w/w) of the applied bronopol and more meaningful, on a molar basis (ratio of molecular weight of bronopol:bromide = 2.5) this percentage would correspond to 43% of the orally applied bronopol.

After intravenous application ([redacted] 1980-A6_02-4) with 10 mg/kg bw of bronopol in rat and rabbit about 85% of the dose was excreted within 24 h. Similar to the situation after oral application, excretion was mainly via urine (74% of dose). Small amounts were seen in expired air (9%) and faeces (1.3%). The main metabolite, NPD, corresponded to 53% of applied dose and no bronopol was detected in urine.



Bronopol 2-nitropropan-1,3-diol (NPD)

Summary:

Toxicokinetics of Bronopol was studied after single and repeated application by the oral and dermal route in males and females of different species, mainly rodents.

In general, Bronopol is rapidly absorbed by the oral and dermal route and most of it excreted via the urine during the first 24 h. Since Bronopol hydrolyses easily under experimental conditions it remains unclear whether Bronopol as such is absorbed and degrades rapidly into its hydrolysis products (metabolites) in the blood stream, or hydrolysis occurs already before absorption. However, the absence of Bronopol in plasma and urine samples of exposed animals indicates that little to no systemic exposure to Bronopol occurs.

The main metabolite detected in urine and plasma samples after oral and dermal application of Bronopol was identified to be NPD.

A difference in the toxicokinetic of Bronopol between males and females of the tested species was not observed in any of the two tested application routes. Following single and repeated oral application of 1–50 mg/kg bw, maximum peak plasma concentrations of the metabolites were

reached within 2 h and a mean half-life time of *ca*. 4 h could be shown for rats and dogs. There was no indication for a potential accumulation in the plasma. The metabolites of Bronopol are rapidly eliminated primarily (>70%) via the urine, whereas excretion via the faeces and exhaled air accounted for less than 10% each and played rather a minor role. Most part of urine excretion (64-81% of the total excretion) happened during the first 24 h after dosing. No significant accumulation of Bronopol metabolites in tissues and organs has been observed. In both tested species (rats and rabbits) fatty tissue, skin and organs involved in excretion, i.e. kidney, liver, and lung showed the highest concentration of residues. Corresponding to the excretion characteristics of the Bronopol derived C-chain approximately 40% of the bromine moiety of orally applied Bronopol was found in the urine.

The percutaneous absorption of Bronopol following single and repeated dermal exposure was examined in rats, rabbits, and mice. Lowest rates for percutaneous absorption of Bronopol has been observed in rats followed by rabbits, where most of the applied dose remained on the dressing and on the skin of the application site. In mice however, more than 80% of the applied dose seemed to be absorbed after 24 h. Since rats are considered the preferred species for animal studies on dermal absorption, these are considered the most relevant and reliable data in the absence of *in vitro* or human data. Therefore, it is concluded that dermal absorption of 50.1% is a scientifically justified, conservative approach for Bronopol (considering the irritant nature of Bronopol for the diluted as well as the neat material).

The release into the plasma was slow and the metabolic pathways for the percutaneously absorbed Bronopol and/or metabolites are similar to those following oral administration, namely rapid metabolism/hydrolysis, main excretion via the urine, no accumulation in the plasma and organs. Unlike after oral exposure the main metabolites of Bronopol NPD was found also in significant amounts in the skin.

A.3.1.2 Values and conclusions used for the risk assessment

Not applicable for the CLH report.

A.3.2 Acute toxicity / STOT SE

A.3.2.1 Acute oral toxicity

Table A-18: Summary table of animal studies on acute oral toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Type of administration (gavage, in diet, other)**	**Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)**	**Value LD50**	**Remarks (e.g. major deviations)**	**Reference**
OECD 401 GLP Rel. 1	Rat Fischer 344 5 sex/group	Bronopol Doses: 100, 150 (females only), 200, 300 (males only) mg/kg bw	Signs of gastrointestinal irritation	LD50 male = 211 mg/kg bw		[redacted] 2001 (A6_01_1-1)

KEY		Administration: single oral application (gavage)	(most of deaths within 24 hours)	LD50 female = 193 mg/kg bw		
OECD 401 GLP Rel. 2	Rat, Sprague-Dawley, male and female, 5 animals/ sex/group	TS: Bronopol (purity not stated) Doses: 250, 500, 1000, and 2000 mg/kg bw Observation period: 14 days Oral Limit-test: 2000 mg/kg bw Range-finding test: 100, 250, 500 and 1000 mg/kg bw Main-test: 250, 500 and 1000 mg/kg bw	Signs of toxicity: hunched posture, piloerection, lethargy, ↓ respiratory rate. Dead animals showed abnormally red lungs	LD50 male = 273 mg/kg bw LD50 female = 354 mg/kg bw LD50 male, female = 305 mg/kg bw	Male rats were more sensitive than the females.	[redacted] 1987 (A6.01.1_01)
Similar to OECD 401 Non-GLP Rel. 2	Rat, Wistar, male and female, 10 animals/sex/group	TS: Bronopol (99.0%) Doses: 200, 280, 390, 550, 770 mg/kg bw Observation period: 7 days Oral	Signs of toxicity: sedation, wheezing, cyanosis, increased salivation, nasal exudate and ataxia. Animals given 550 or 770 mg/kg bw also had slow or laboured respiration, and two females became prostrate.	LD50 male = 307 mg/kg bw LD50 female = 342 mg/kg bw	Male rats were more sensitive than the females. Study was conducted before OECD TG 401 was available and GLP was compulsory.	[redacted] 1992 (A6.01.1_02)
Non-guideline Non-GLP Rel. 2	Rat, Wistar, male, 10 animals/group	TS: Bronopol (93.1%; impurities: sodium bromide 3.0%, 2-methyl-2-nitropropane-1,3-diol 0.6%, tris(hydroxymethyl)nitromethane 0.3%) Doses: 36, 54, 80, 120, 180, 270, 400, 600 mg/kg bw Observation period: 5 days	Signs: wheezing, gasping, nasal exudate, laboured respiration. Some animals were inactive and adopted a hunched body position.	LD50 male = 254 mg/kg bw	Study was conducted before OECD TG 401 was available and before GLP was compulsory.	[redacted] 1992 (A6.01.1_03)

		Oral	Necropsy of dead animals revealed gastrointestinal irritation; adrenals appeared enlarged and darkened in some animals of the highest dose groups.			

No human data on acute oral toxicity is available.
No other studies relevant for acute oral toxicity are available.

A.3.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Bronopol is harmful when applied orally in a single dose. Acute oral toxicity caused by the substance is related to severe haemorrhage and ulceration of the gastrointestinal tract and gross pathological alterations of liver, kidneys, and lungs. Under test conditions of the OECD 401 application of Bronopol of unknown purity in doses ranging from 250 to 2000 mg/kg bw to male and female rats the LD50 was reported to be 305 mg/kg bw with male rats being slightly more sensitive than female rats ([redacted] 1987; A6.01.1_01). At all tested doses mortalities occurred within one day after application of the substance and all treated animals suffered from symptoms indicative of generalized toxicity as they were hunched posture, piloerection, lethargy and decreased respiratory rate. Surviving animals recovered fully during the observation period of 14 days and were reported to show no signs of toxicity. The extent and kind of pathological findings after necropsy were indicative of a severe irritating effect of Bronopol at doses ≥ 250 mg/kg bw. Animals that died during the study revealed dark livers and kidneys, severe haemorrhage or ulceration of the gastric mucosa and haemorrhage of the small and large intestines. In addition, increased incidence of abnormally red lungs, dark spleens and sloughing of the non-glandular region of the stomach were noted in animals treated with high doses of Bronopol.

These results are consistent with two studies conducted prior to the existence of OECD 401 testing Bronopol of known purity, which indicate little to no impact of the purity of the tested substance on the acute oral toxicity. For pharmaceutical grade Bronopol applied to male and female rats at doses ranging from 200 to 770 mg/kg bw the LD50 reported for male rats (307 mg/kg bw) was lower than the LD50 reported for female rats (342 mg/kg bw) ([redacted] 1992; A6.01.1_02). Most deaths occurred within 19 hours after dosing, but some occurred up to 72 hours. The signs of toxicity included sedation, wheezing, cyanosis, increased salivation, nasal exudate and ataxia. Animals given 550 or 770 mg/kg bw also had slow or laboured respiration, and two females became prostrate. No gross abnormalities were observed at necropsy of decendents or animals sacrificed at the end of the study.

Only males were tested for technical grade bronopol showing LD_{50} of 254 mg/kg bw ([redacted] 1992; A6.01.1_03). Deaths occurred within five days after dosing. Overt signs of toxicity, including wheezing, gasping, nasal exudate, laboured respiration, were seen after dosing

with 80 mg/kg bw or more. Necropsy of the animals that died during the observation period revealed signs of irritation of the gastrointestinal tract and gross pathological alteration of liver, adrenals, and spleen and necropsy of the animals that were sacrificed at the end of the observation period revealed a single case of small spleen seen at 400 mg/kg bw.

In an acute oral toxicity test according to OECD test guideline 401 in rats oral LD50 values of 193 mg/kg bw (females) and 211 mg/kg bw (males) were determined for Bronopol ([redacted] 2001; A6_01_1-1). In this acute oral toxicity study most of deaths occurred within the first day after treatment. In the rats that died various combinations of dark glandular mucosa of the stomach, dilatation of the stomach with cloudy fluid, perineal soiling, and hemolyzed blood in the gastrointestinal tract were seen. At 300 mg/kg bw two additional males had dark foci in the lungs. In surviving animals clinical signs comprised lacrimation, noisy respiration, decreased responsiveness to touch, decreased activity, decreased reactivity to handling, soft feces, vocalization, and soiling of the perioral, perinasal, perineal, and/or periocular regions except for 150 mg/kg bw treated females, which revealed no sign of treatment. However, no gross lesions were apparent during post mortem examination of these surviving animals and all of them returned to normal by at day 9 latest.

Based on the results of the acute oral toxicity studies available for Bronopol an oral LD50 of < 300 mg/kg was determined. The most conservative oral LD50 values are 211 mg/kg bw (males) and 193 mg/kg bw (females). Therefore, Bronopol is classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008.

A.3.2.1.2 Comparison with the CLP criteria

Bronopol should be classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008 (50 < LD50 ≤ 300 mg/kg bw). Bronopol has an entry in Annex VI to CLP as Acute tox Cat. 4 H302 under Regulation (EC) No 1272/2008 (ATP 1 to CLP). However, as the most conservative values, from a reliable guideline study are below the cut-off value for classification in Category 4, a higher, more severe classification is triggered.

A.3.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Bronopol has an entry in Annex VI to CLP as Acute tox Cat. 4 H302 under Regulation (EC) No 1272/2008 (ATP 1 to CLP). Nevertheless, based on the available data, the oral LD50 values are values are 211 mg/kg bw (males) and 193 mg/kg bw (females) and thus, Bronopol should be classified as Acute tox Cat.3 H301 under Regulation (EC) No 1272/2008. An ATE_{oral} = 193 mg/kg bw should be established based on the lowest LD50 obtained.

A.3.2.1.4 Conclusion on acute oral toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.2 Acute dermal toxicity

Table A-19: Summary table of animal studies on acute dermal toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Surface area	Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)	Value LD50	Remarks (e.g. major deviations)	Reference
OECD 402 **GLP** **Rel. 1** **KEY**	Rat Wistar 5 sex/group	Bronopol Dose: 2000 mg/kg bw single dermal application	2/5 females died. And showed discolorations of adrenal glands Clinical signs: dermal irritation	LD50 >2000 mg/kg bw		[redacted] 2000 (A6_01_2-1)
OECD 402 **GLP** **Rel. 1**	Rat, Wistar, male and female, 10 animals/sex/group	TS: Bronopol (99.7%) Dose: 2000 mg/kg bw Exposure: 24 hours, semi-occlusive Observation period: 18 days Dermal	Signs of general toxicity (poor general state, dyspnoea and apathy), and cutaneous effects at the application site (white discoloration of the skin, erythema, edema, eczema-like skin change, scaling and crust formation). Skin showed incrustation and full thickness necrosis in 4/5 males and 5/5 females.	LD50 male and female > 2000 mg/kg bw		[redacted] 2000 (A6.01.2_01)

Audited KEY	Male Boots-Wistar rats, 10 animals	Main study: Groups of ten male rats. Dose: 25, 100, 400 and 1600 mg/kg, respectively. Observation period: 14 days	Animals at all dosages: damage to the treated area of skin dark perianal stains 1 rat given 100 mg/kg had diarrhoea. Animals given 25 mg/kg: mild to moderate eschar formation, while those Animals given 100 mg/kg or more: grey/white areas that progressed to moderate to severe eschar formation. 3 rats given 1600 mg/kg died. Macroscopic examination revealed evidence of mild gastrointestinal irritation and orange-coloured lungs.	1600 mg/kg bw		[redacted] 1992 (A6.01.2_02)

No guideline	Rats 2 male/group	64, 160, 400 and 1000 mg/kg bw in acetone	All rats given 1000 and 4000 mg/kg died overnight. Rats given 160 mg/kg: yellow staining of the skin, edema in the skin on the flanks. The rats became cold, prostrate and respiration was laboured and died on the second and sixth days after treatment. Autopsy revealed congestion of the lungs. 64 mg/kg: yellow staining of the skin and slight scabbing in the week after dosing.	Cannot be determined due to the small number of animals per group. Between 64 – 160 mg/kg bw		[redacted] 1967 (A6.01.2_03)

No human data on acute dermal toxicity is available.

No other studies relevant for acute dermal toxicity are available.

A.3.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Acute dermal toxicity for Bronopol (purity 99.7%) is low when tested in rats according to OECD test guideline 402. When applied in 0.5% aqueous Tylose® at a dose of 2000 mg/kg bw for 24 hours under semi-occlusive conditions no mortality occurred, thus resulting in a dermal LD_{50} of >2000 mg/kg bw ([redacted] 2000; A6_01_2-1). Treated animals of both sexes showed signs of general toxicity, namely poor general state, dyspnoea and apathy. Clinical symptoms of the treated animals appeared during the first day after application of the test substance but after removal of the test substance the animals recovered during the following 24 hours. Cutaneous effects at the application site were seen in both, males and females, and were described as white discoloration of the skin, erythema, edema, eczema-like skin change, scaling and crust formation. These effects lasted until the end of the observation period (18 days). Necropsy revealed incrustation and full thickness necrosis seen at the application site in all but one tested animal, thus indicating low acute systemic toxicity when applied to the skin.

The report A6.01.2_02 ([redacted], 1992) was conducted to investigate the effects of a single dermal application of technical-grade bronopol in rats. In a preliminary study, groups of two rats received applications of bronopol at 50, 100, 200, 400, 800 or 1600 mg/kg, as a solution in distilled water. In the main study, groups of ten rats received dosages of bronopol at 25, 100, 400, or 1600 mg/kg. The treated areas were covered with occlusive dressings for 24 hours, then the dressings were removed and the exposed areas were cleaned. The rats were observed for a 14-day period, and were dissected and examined when they died or were killed at the end of the study. In the preliminary study, both rats treated with 1600 mg/kg died during the exposure period. Evidence of irritation and damage to the treated skin was seen in the survivors at all dosages. Macroscopic examination of decedents revealed evidence of mild gastrointestinal irritation and orange-coloured lungs. In the main study, animals at all dosages had dark perianal stains at the end of the treatment period, and one rat given 100 mg/kg had diarrhoea. Damage to the treated area of skin was seen at all dosages; animals given 25 mg/kg had mild to moderate eschar formation, while those given 100 mg/kg or more had grey/white areas that progressed to moderate to severe eschar formation. Three rats treated with 1600 mg/kg died during the exposure period. Macroscopic examination revealed evidence of mild gastrointestinal irritation and orange-coloured lungs. The results of the present study show that bronopol in aqueous solution had a dermal LD_{50} of about 1600 mg/kg.

A report presented A6.01.2_03 ([redacted], 1967) bronopol in solution in acetone was administered percutaneously to groups of two male rats which were observed for 3 weeks after dosing. No signs of toxicity were observed for the first four hours after treatment. All rats given 1000 and 4000 mg/kg bw died overnight. The skin was stained yellow and there was extensive subcutaneous edema and haemorrhage. One rat had slight congestion of the mucose in the secretory stomach. The rats given 160 mg/kg bw had yellow staining of the skin and edema was palpable in the skin on the flanks from the day after treatment. The rats became cold, prostrate and respiration was laboured and they died on the second and sixth days after treatment. Autopsy confirmed the presence of subcutaneous edema and also revealed congestion of the lungs. 64 mg/kg bw produced yellow staining of the skin and slight scabbing in the week after dosing. Otherwise, there were no effects and when killed 3 weeks after dosing postmorten appearance was normal. On the basis of the small number of animals used in this test, bronopol has a dermal LD_{50} of between 64 and 160 mg/kg bw.

Low acute toxicity for Bronopol (purity 98.7 %) was also observed in another acute dermal toxicity test according to OECD test guideline 402 ([redacted] 2000; A6_01_2-1). Two out of five treated females died on days 2 to 3 and their necropsy showed a discoloration of the adrenal glands. In another female decreased motility and reactivity, laboured breathing and piloerection was observed on days 2 and 3. In both sexes the following skin reactions were observed: reddening, formation of scale, encrustations, yellow and grey discolorations. The LD_{50} was >2000 mg/kg bw.

Based on the results of the acute dermal toxicity studies available for Bronopol, both studies A6_01_2-1 ([redacted] 2000) with a dermal LD50 of > 2000 mg/kg and A6.01.2_02 ([redacted], 1992) with a dermal LD50 of 1600 mg/kg, showed signs of general toxicity and mortality occurred. For the classification, the worst case value of LD50 (1600 mg/kg) is considered. Therefore, Bronopol is classified as Acute tox Cat. 4 H312 under Regulation (EC) No 1272/2008.

A.3.2.2.2 Comparison with the CLP criteria

Bronopol is classified as Acute tox Cat. 4 H312 under Regulation (EC) No 1272/2008 (1000 < LD50 ≤ 2000 mg/kg). Bronopol has a minimum classification as category 4. Classification in this category is consistent with the data presented above and, therefore, we propose category 4 for acute dermal toxicity and removal of the * denoting minimum classification.

A.3.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Bronopol has an existing harmonised classification as Acute tox Cat. 4*; H312 under Regulation (EC) No 1272/2008, which is supported by the data presented in this CLH report. In addition, an ATE_{dermal} = 1600 mg/kg bw should be added based on the lowest LD50 obtained in the study presented above.

A.3.2.2.4 Conclusion on acute dermal toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.3 Acute inhalation toxicity

Table A-20: Summary table of animal studies on acute inhalation toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body/ head only)**	**Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)**	**Value LC50**	**Remarks (e.g. major deviations)**	**Reference**
OECD 403 **GLP** **Rel. 2** **KEY**	Rat Fischer 344 5 sex/group	Bronopol Concentrations: 120, 1140 mg/m³ Exposure: 4 hours (inhalation, nose only)	In the high dose level of 1140 mg/m³ (with a transient concentration of 3640 mg/m³):	120 mg/m³ <LC50 <1140 mg/m³	Significant difficulty in maintining a stable chamber aerosol concentration	[redacted] 2003 (A6_01_3-1)

			10/10 died. Signs of respiratory irritation		during the 1140 mg/m³ exposure	
Similar to OECD 403 **Non-GLP** **Rel. 2** **KEY**	Rat, Sprague-Dawley, male and female, 5 animals/ sex/group	TS: Bronopol (99.7%) Nominal concentrations: 0, 1.80, 2.59, 23.23 mg/L Analytical concentrations: 0, 0.038, 0.089, 0.588 mg/L. Exposure: 4 hours Inhalation	One male rat of the 0.588 mg/L group died whereas two further animals of the same test group (one male and one female) were sacrificed for humane reasons as they suffered from inflammation of the eyes. All animals of the remaining groups survived. Nearly all animals of the 0.588 mg/L group suffered from nasal discharge, red staining and inflammation of the eyes and staining of the head; sometimes these symptoms were further accompanied by a swelling of the head, throat and/or the forepaws. These effects were consistent with local irritation of those areas, which came in	LC50 for males and females > 0.588 mg/L (equivalent to > 588 mg/m³)	Study was conducted before GLP was compulsory.	[redacted] 1986 (A6.01.3_01)

			direct contact with the test substance.			

No human data on acute inhalation toxicity is available.

No other studies relevant for acute inhalation toxicity are available.

A.3.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Dust of Bronopol (purity 99.7%) showed little acute toxicity by inhalation when tested under conditions similar to OECD test guideline 403. When Sprague-Dawley rats were exposed nose-head to measured concentrations of 0.038 to 0.588 mg/L for four hours, no treatment related mortality occurred, thus the LC50 for Bronopol in rats was determined to be >0.588 mg/L/4 h ([redacted] 1986-A6.01.3_01). One case of mortality was occurred within 24 hours after exposure with the highest tested concentration of 0.588 mg/l and two animals were sacrificed 24 hours after exposure for animal welfare reason because they suffered from inflammation of the eyes. Detailed macroscopic examination of the cranial cavity and respiratory tract as well as the histopathological examination of the lungs, liver, kidneys, and skin revealed no treatment related cause of death. Clinical signs observed in the high dose group consisted in nasal discharge, staining of the head and inflammation of the eyes. These effects were partly accompanied by swelling of the head, throat and forepaws. Except for one animal, clinical signs had generally disappeared by the third day of the observation period. No clinical symptoms were observed in the low dose group, in animals of the mid dose group hunched posture and piloerection were observed for the first 24 hours. The NOEC was determined to be 0.038 mg/l/4h. Macroscopic and histopathological examination was inconspicuous in these two groups. In conclusion, the reported signs of toxicity after inhalation exposure to Bronopol were consistent with local irritation of those areas in direct contact with the compound rather than systemic effects. The LC50 value from this study (0.588 mg/L/4h), according to Regulation (EC) No 1272/2008, corresponds to a classification as Acute tox Cat. 3 H331.

Another acute inhalation toxicity study was conducted according to OECD 403 exposing Fisher 344 rats to Bronopol at 120 and 1140 mg/m³ (nose only) for 4 hours ([redacted] 2003-A6_01_3-1). At 1140 mg/m^3 4/5 males and 3/5 females died during exposure and the remaining animals died until end of day 3. At 120 mg/m^3 only one male rat died. Significant difficulty in mainting a stable chamber aerosol concentration was encountered during the 1140 mg/m^3 exposure (with a transient concentration of 3640 mg/m^3). The test material tended to agglomerate, forming larger multimeric particulates at chamber concentrations approximately 1 mg/m^3 and above, resulting in a relatively high mean MMAD and geometric standard deviation. The observed clinical signs additionally indicate a potential for respiratory irritation.

Taken together, the inhalation LC50 value lies between 0.588 mg/L and 1.14 mg/L.Therefore, Bronopol is classified as Acute tox Cat. 3 H331 under Regulation (EC) No 1272/2008.

A.3.2.3.2 Comparison with the CLP criteria

Bronopol should be classified as Acute tox Cat. 3 H331 (dust/mist) under Regulation (EC) No 1272/2008 (0.5 < LC50 ≤ 1.0 mg/L for dusts and mists).

A.3.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available data, the inhalation LC50 value is determined to lie between 0.588 and 1.140 mg/L and thus, Bronopol should be classified as Acute tox Cat.3 H331 (dust/mist) under Regulation (EC) No 1272/2008. An $ATE_{inhalation}$ = 0.588 mg/L (dust/mist) should be established based on the lowest concentration in the derived range.

A.3.2.3.4 Conclusion on acute inhalation toxicity related to risk assessment

Not applicable for the CLH report.

A.3.2.4 Specific target organ toxicity – single exposure Category 1 and 2 (STOT SE 1 and 2)

No data addressing specific target organ toxicity after single exposure (STOT SE 1 and 2) is available.

No human data addressing specific target organ toxicity after single exposure (STOT SE 1 and 2) is available.

No other studies relevant for specific target organ toxicity after single exposure (STOT SE 1 and 2) are available.

A.3.2.4.1 Short summary and overall relevance of the provided information on STOT SE 1 and 2

No studies addressing specific target organ toxicity in animals or humans are available. Studies on acute toxicity do not indicate any specific target organ. In addition, the available repeated dose toxicity studies do not provide any consistent evidence for a specific target organ toxicity after single exposure. Thus, no NOAEL and LOAEL can be derived for STOT SE, and classification and labelling for STOT SE 1 or 2 according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.2.4.2 Comparison with the CLP criteria

No studies addressing specific target organ toxicity in animals or humans are available. Studies on acute toxicity do not indicate any specific target organ. In addition, the available repeated dose toxicity studies do not provide any consistent evidence for a specific target organ toxicity after single exposure. Thus, no NOAEL and LOAEL can be derived for STOT SE, and classification and labelling for STOT SE 1 and 2 according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.2.4.3 Conclusion on classification and labelling for STOT SE 1 and 2

No studies addressing specific target organ toxicity in animals or humans are available. Studies on acute toxicity do not indicate any specific target organ. In addition, the available repeated dose toxicity studies do not provide any consistent evidence for a specific target organ toxicity after single exposure. Thus, no NOAEL and LOAEL can be derived for STOT SE, and no classification and labelling for STOT SE 1 or 2 according to CLP criteria should be required under Regulation (EC) No 1272/2008.

A.3.2.5 Specific target organ toxicity – single exposure Category 3 (STOT SE 3)

Table A-21: Summary table of animal studies on STOT SE 3

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	Results (including type of effect; respiratory tract irritation or narcotic effects)	Remarks (e.g. major deviations)	Reference
OECD 403 **GLP** **Rel. 2** **Supportive**	Rat Fischer 344 5/sex/group	Bronopol (99.4%) Dose levels: 120, 1140 mg/m³ Exposure: 4 h (inhalation, nose only)	Clinical signs during exposure included bloody nose and mouth breathing (at 1140 mg/m³ only) as well as labored breathing, and soiling of the hair coat (both concentrations). At the high concentration the observed gross nasal lesions and impaired respiration may be related to the deposition of the relatively large particles of Bronopol in the upper respiratory tract. Clinical signs after exposure included combinations of slow, noisy, deep and/or labored respiration; perinasal, perioral, perineal, abdominal and/or extensive body soiling; and swelling of the muzzle and chin.	High initial concentration (3640 mg/m³)	[redacted] 2003 A6_01_3-1
Similar to OECD 403 **Non-GLP** **Rel. 2**	Rat, Sprague-Dawley, male and female, 5 animals/ sex/group	Bronopol (99.7%) Dose levels: 0, 1.80, 2.59, 23.23 mg/L	Nearly all animals of the 0.588 mg/L group suffered from nasal discharge, red staining and		[redacted] 1986 A6.01.3_01

Key		(nominal) 0, 0.038, 0.089, 0.588 mg/L (analytical) Exposure: 4 h (inhalation, Nose-Head only)	inflammation of the eyes and staining of the head; sometimes these symptoms were further accompanied by a swelling of the head, throat and/or the forepaws. These effects were consistent with local irritation of those areas, which came in direct contact with the test substance. These symptoms mainly were seen on the day of exposure and disappeared within 3 days; in some case, staining of the head reappeared at the end of the observation period. In one case (female of the 0.588 mg/L group) the marked staining persisted throughout the whole observation period and was accompanied by sores, fissuring and desquamation of the head skin during the second week of observation.		

No human data addressing specific target organ toxicity after single exposure (STOT SE 3) is available.

No other studies relevant for specific target organ toxicity after single exposure (STOT SE 3) are available.

A.3.2.5.1 Short summary and overall relevance of the provided information on STOT SE 3

In the available acute inhalation toxicity studies, the observed clinical signs, such as bloody nose and mouth breathing, slow, noisy, deep and/or labored respiration, nasal discharge as well as swelling of the head and throat were considered indicative of respiratory irritation ([redacted] 2003-A6_01_3-1 and [redacted] 1986-A6.1.3_01).

A.3.2.5.2 Comparison with the CLP criteria

The observed clinical signs from the available acute inhalation toxicity studies are indicative of respiratory irritation. Therefore, criteria for classification of Bronopol as single target organ toxicity – single exposure (STOT SE) Cat. 3 are met. Bronopol has an entry in Annex VI to CLP as STOT SE Cat. 3 H335 under Regulation (EC) No 1272/2008 (1st ATP to CLP).

A.3.2.5.3 Conclusion on classification and labelling for STOT SE 3

Due to the available data presented, the existing harmonised classification as STOT SE 3 is warranted. Therefore, the classification as STOT SE 3 should be retained.

A.3.2.5.4 Overall conclusion on acute toxicity related to risk assessment

Not applicable for the CLH report.

A.3.3 Skin corrosion and irritation

No *in vitro* studies on skin corrosion/irritation are available.

Table A-22: Summary table of animal studies on skin corrosion/irritation

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**Results Average score for erythema/eschar and oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility, other adverse local/systemic effects, histopathological findings**	**Remarks (e.g. major deviations)**	**Reference**
OECD 404 **GLP** **Rel. 1**	Rabbit Himalayan 3 males	Bronopol (98.7%) Vehicle: aqua ad iniectabilia Dose level: approx. 500 mg Bronopol per animal (6 cm^2) Duration of exposure: 4 hours Postexposure period: 72 hours	Erythema (24h, 48h, 72h): 0 Edema (24h, 48h, 72h): 0 Reversibility: n.a. Result: non-irritant		[redacted] 2000a (A6_01_4-1)

OECD 404 **GLP** **Rel. 2** **KEY**	Rabbit New Zealand White 6 animals (sex not specified)	Bronopol Vehicle: distilled water Dose level: 0.5 g Bronopol moistened with 0.5 mL distilled water (1 g/mL) per animal Duration of exposure: 4 hours Postexposure period: 14 days	Erythema 1 h: 2.5 24 h: 3.3 48 h: 3.7 72 h: 3.5 7 days: 3.3 14 days: - Edema 1 h: 2.8 24 h: 3.7 48 h: 2.7 72 h: 2.0 7 days: 1.5 14 days: no edema Result: irritating to skin of rabbit		[redacted] 1987 (A6.01.4_01)

Table A-23: Summary table of human data on skin corrosion/irritation

Type of data / report, Reliability, Key / supportive study	**Test substance (including purity), Vehicle**	**Relevant information about the study**	**Main effects, Observations**	**Reference**
Study with volunteers	Bronopol 1% aqueous Bronopol solution	129 patients received patch tests to a standard screening battery containing more than 30 commonly encountered allergens; Patches were placed on the back of the patients and removed after 48 hours. Readings were performed after different time points after start of application.	Of the 129 patients, 57 were tested with 1% aqueous Bronopol only, 23 subjects showed irritant reactions to 1 % Bronopol.	[redacted] 1983 (A6_01_5-4, A6.12.6_03)
Study with volunteers	Bronopol 1% aq. solution	Patch testing was conducted in 190 patients with contact dermatitis	25 subjects showed positive skin reaction to Bronopol at 1 % in aq., The concentration chosen for patch testing could have caused irritant reactions and are too close to the irritancy threshold to give a clear distinction between irritancy	[redacted] 1977 (A6.12.6_04)

			and sensitisation.	
Study with volunteers	Bronopol Preliminary test: 0.1, 0.5, 1.0, 2.5 and 5.0 % Bronopol in paraffin Main test: Induction was conducted with 5.0% Bronopol in paraffin (0.5 g). Challenge was conducted with 0.25% Bronopol in paraffin (0.5 g).	Patch testing (closed patch test conditions) was conducted in 8 normal subjects (Preliminary test) and 120 normal subjects for induction (Main test), therefrom 93 subjects for challenge: Preliminary test: 1 application daily over 21 days (i.e. 21 applications at intervals of 24 hrs) Main test: 10 applications over a period of 3 weeks (48 hrs interval during week, 72 hrs interval during weekend.)	Preliminary test: The irritancy threshold was about 0.5 to 1% Bronopol in paraffin. Main test: Following induction with 5% Bronopol in paraffin, several cases of skin irritation were seen. Irritancy threshold was approximately 0.5-1 %.	[redacted] 1977 (A6_01_5-3, A6.12.6_02)
Study with volunteers	Bronopol 1% in pet. 0, 0.5, 1 and 2 % Bronopol in pet., and 0, 0.05, 0.1 and 0.25 % in aqueous buffer at pH 5.5.	Patch testing was conducted in 149 patients attending a contact dermatitis clinic	Bronopol (1 % in pet.) showed slight erythema in 2/10 patients, at 2 % in pet. moderate erythema in 4/10, and at 0.25% in aq. 1/10 slight erythema.	[redacted] 1978 (A6.12.6_01)
Medical surveillance data on manufacturing plant personnel/ Survey	Bronopol Saturated aqueous solutions or powder of Bronopol	In the 1980's, a survey was conducted of the medical records of staff employed in the manufacture of Bronopol in the UK. Report on data on occupational exposure of 50 workers to Bronopol, during an 8-year period.	Incidences of rashes or superficial burns due to exposure to crystalline Bronopol or its aqueous solution were reported by 23 men. Of these, 8 reported a second occurrence, 6 a third and 3 a fourth. Most incidents have arisen as a result of breakdown of protective measures. There is no record of any individual having to give up work. In addition, no reports have been received from the workers own private doctors to suggest any incidence of serious respiratory or renal disease as a result of	Anonymous 1984 (B6.6_01)

			exposure to Bronopol.	

A.3.3.1 Short summary and overall relevance of the provided information on skin corrosion / irritation

Results of an available non-detailed skin irritation study ([redacted] 2000a; A6_01_4-1) in 3 male rabbits performed under guidelines showed no skin irritation after dermal application of Bronopol to rabbits. On the contrary to these study results, results of another detailed skin irritation study with Bronopol showed clear skin irritation effects ([redacted] 1987; A6.01.4_01). In this study, Bronopol of unknown purity was tested for acute dermal irritation in six New Zealand White rabbits under semi-occlusive conditions according to OECD 404. The test material was prepared by moistening the test substance with distilled water and applied under semi-occlusive conditions. Five of six rabbits developed severe erythema with green/brown coloured necrosis, which did not reverse until the end of the observation period. Four of six rabbits developed severe edema extending beyond the application site, which reversed fully within 14 days after application of the test substance. Moreover, at the end of the observation period eschar, desquamation, and signs of tissue destruction were reported, however, the occurrence of full thickness necrosis was not stated. It seems unlikely that the reported severe skin irritation effects of Bronopol were caused by impurities of the test substance (its impurity was not stated) but rather by hydrolysis products during the preparation of the test substance in unbuffered distilled water. Bronopol is known to degrade considerably in aqueous solution at pH 7 within four hours ([redacted] 1996 and [redacted] 1991, A7.1.1.1.1_01a and b) resulting in the formation of highly irritating/corrosive substances and intermediates such as glycolic acid, formic acid and formaldehyde. Cutaneous reactions observed in this study may therefore overestimate the skin irritancy of neat Bronopol.

Nevertheless, the results of this positive skin irritation study are supported by human data. Most of the available publications are studies with volunteers which were subjected to patch testing with Bronopol. In each study some of the participants ([redacted] 1983 (A6_01_5-4, A6.12.6_03), [redacted] 1977 (A6.12.6_04), [redacted] 1977 (A6_01_5-3, A6.12.6_02), [redacted] 1978 (A6.12.6_01)) revealed signs of skin irritation when exposed to Bronopol at 0.5 – 1% (at the induction phase). Additionally, medical surveillance data (Anonymous 1984; B6.6_01), a survey of medical records of staff employed in the manufacture of Bronopol in the UK, showed incidences of rashes or superficial burns due to exposure to crystalline or aqueous solution during incidents in which the protective measures were broken.

A.3.3.2 Comparison with the CLP criteria

Based on the more stringent results of a skin irritation study in rabbits (5/6 animals with score ≥ 2,3 and < 4,0 for oedema and erythema) and records of skin irritation from human data, Bronopol is considered as skin irritating to the skin. Furthermore, Bronopol has an entry in Annex VI to CLP as Skin irritant Cat. 2 H315 under Regulation (EC) No 1272/2008 (1st ATP to CLP).

A.3.3.3 Conclusion on classification and labelling for skin corrosion / irritation

Due to the available data presented, the existing harmonised classification as Skin Irrit. 2 is warranted. Therefore, the classification as Skin Irrit. 2 should be retained.

A.3.3.4 Overall conclusion on skin irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.4 Serious eye damage and Eye irritation

No *in vitro* studies on serious eye damage and eye irritation are available.

Table A-24: Summary table of animal studies on serious eye damage and eye irritation

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**Results Average score for corneal opacity, iritis, conjunctival redness and conjunctival oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility**	**Remarks (e.g. major deviations)**	**Reference**
OECD 405 **GLP** **Rel. 1**	Rabbit Himalayan 1 male	Bronopol (98.7%) Vehicle: none Dose levels: 100 mg/eye Duration of exposure: Eyes remained unwashed Postexposure period: 21 days	Cornea: Grade 4 opacity at 1h after application, further assessment was not possible (whitish deposits (probably pus) from 72h onwards, cornea destroyed within 18 days) Iris: Grade 2 irritation at 1h after application, further assessment was not possible Conjunctival redness (mean) 24h, 48h, 72h: 1.3 Conjunctival chemosis (mean) 24h, 48h, 72h: 3.3 Reversibility: no Result: Risk of serious damage to eye		[redacted] 2000b (A6_01_4-2)

Draize Test **Non-GLP** **Rel. 2**	Rabbit New Zealand White 3 females/group	Bronopol Vehicle: polyethylene glycol 400 Dose levels: 0.5, 2 and 5% solution Exposure duration: 24h Postexposure period: 21 days	Cornea 24h, 48h, 72h: 0 Iris 24h, 48h, 72h: 0 Conjunctive 24h: 2.0 48h: 1.7 72h: 1.0 Chemosis 24h: 2.3 48h: 0.3 72h: 0.3 Reversibility: yes, within 14 days Result: irritating (5% solution)		[redacted] 1996 (A6.01.4_02)
Similar to OECD 403 **Non-GLP** **Rel. 2**	Rat	Bronopol (99.7%) Dose levels: 0, 0.038, 0.089 and 0.588 mg/L Exposure via nose-head only	Severely irritating to the eyes at 0.588 mg/L		[redacted] 1986 (A6.01.3_01)

No human data on serious eye damage/eye irritation is available.

A.3.4.1 Short summary and overall relevance of the provided information on serious eye damage / eye irritation

Results of an eye irritation study performed similarity to OECD 405 ([redacted] 2000b; A6_01_4-2) revealed marked effects after application of Bronopol to eyes of rabbits. Grade 4 corneal opacity and grade 2 iris reactions were observed at 1 hour after application and by day 18 complete destruction of the cornea became apparent. In addition, slight to moderate conjunctival redness and moderate to marked conjunctival chemosis were recorded. Eye reactions were not reversible within 21 days after treatment.

In another study, similar to the Draize test ([redacted] 1996; A6.01.4_02) Bronopol was tested in 0.5%, 2% and 5% solution in polyethylene glycol 400 (PEG 400). PEG 400 itself caused slight eye irritation, however less pronounced than after instillation of Bronopol at the highest concentration into the eyes. It is concluded that Bronopol dissolved in PEG 400 is irritant to the rabbit eye at 5% but not as 2% or 0.5%.

Results of acute inhalation study ([redacted] 1986; A6.01.3_01) supported the results above, indicating an eye irritation potential of Bronopol. Rats which were exposed to respirable Bronopol particles exhibited severe eye irritation after 4 hours of exposure. In two cases, animals were scarified for welfare reason due the severity of the ocular reaction.

A.3.4.2 Comparison with the CLP criteria

Based on the findings from eye irritation studies performed according or similarly to OECD 405 / Draize method and an acute inhalation study of Bronopol an eye irritating potential can be concluded. Bronopol has an entry in Annex VI to CLP as Eye damage Cat. 1 H318 under Regulations (EC) No 1272/2008 (1st ATP to CLP).

A.3.4.3 Conclusion on classification and labelling for serious eye damage / eye irritation

Due to the available data presented, the existing harmonised classification as Skin Dam. 1 is warranted. Therefore, the classification as Eye Dam. 1 should be retained.

A.3.4.4 Overall conclusion on eye irritation and corrosivity related to risk assessment

Not applicable for the CLH report.

A.3.5 Skin sensitisation

Table A-25: Summary table of animal studies on skin sensitisation

Method, Duration of study, Route of exposure (e.g. topical / intradermal, induction / challenge if relevant), Guideline, GLP status, Reliability, Key / supportive study	**Species, Strain, Sex, No / Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**Results (e.g. EC3-value or amount of sensitised animals at induction dose)**	**Remarks (e.g. major deviations)**	**Reference**
OECD 406 Maximisation test GLP Rel. 1 KEY	Guinea pigs Hsd Poc:DH 10 females (treated), 5 females (control)	Bronopol (98.7%) Vehicle: polyethylene glycol 400 (PEG400) Dose levels:	Number of animals sensitised/total number of animals: 0/10 (treated) 0/5 (control)		[redacted] 2001 (A6_01_5-1)

		Induction: intradermal 1% Bronopol in PEG 400, epidermal 25% Bronopol in PEG 400 Challenge: 12% Bronopol in PEG 400 day 1 (intradermal induction) day 8 (epidermal induction) day 22 (challenge) scoring 24h, 48h after challenge	Non-sensitising		
OECD 429 **Local lymph node assay (LLNA)** **GLP** **Rel. 1** **KEY**	Mice BALB/cAnNCrl 6 females/group	Bronopol (99.7%) Vehicle: DMSO Dose levels: Induction: Topical: Application of 25 µL/ear on the dorsal surface of both ears at 0% (vehicle), 0.4%, 2%, 10% or 50% Bronopol in DMSO on three consecutive days, based on pre-test. Pre-test: 1%, 5%, 20%, 40% and 80% Bronopol in DMSO (25 µL/ear on two consecutive days).	Stimulation index <3 at 0.4%, 2% and 10% Bronopol (Stimulation Indexes (SI) were respectively, 1.6-, 2.4-, and 2.4- fold greater than vehicle controls) (in DMSO) Non-sensitising		[redacted] 2005 (A6_01_5-2)
Guinea pig maximization test similar to OECD 406 **Non-GLP** **Rel. 2**	Guinea pig	Bronopol (100%) Dose levels: Induction: used as 0.02% (w/v; injection induction; solvent:	After 3 challenges with Bronopol (0.4% aqueous solution), 2/10 animals were sensitised.		[redacted] 1976 (A6.01.5_01)

KEY		0.9% physiological saline) and 1.5% (w/v; application induction; solvent: distilled water) solutions. Challenge: used as 0.4% (w/v); solvent: distilled water.	Sensitisation was not reduced at 0.2% Bronopol (aqueous solution), tested in a fourth challenge. 0/9 animals were sensitised after cross-challenge with formaldehyde. Bronopol is not a skin sensitiser and sensitisation to formaldehyde was ruled out as well.		

Table A-26: Summary table of human data on skin sensitisation

Type of data / report, Reliability, Key / supportive study	**Test substance (including purity), Vehicle**	**Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)**	**Main effects, Observations**	**Reference**
Study with volunteers, **KEY STUDY**	Induction was conducted with 5.0% Bronopol in paraffin (0.5 g), an irritant concentration. Challenge was conducted with 0.25% Bronopol in paraffin (0.5 g).	Humans 93 healthy human volunteers 10 epidermal inductions in 3 weeks epidermal challenge 2 weeks after last induction	0/93 (no. sensitised/total no.) Non-sensitising Skin irritation after induction with 5%	[redacted] 1977 (A6_01_5-3, A6.12.6_02) (reported in Attley 1977 (B6.7_04))

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)	Main effects, Observations	Reference
Study with volunteers, **KEY STUDY**	Challenge with 0.25-0.5% Bronopol; Challenge with 1% Bronopol not considered relevant as irritant concentration	Humans Patients in a tertiary care centre with localized or general dermatitis; two groups: 57 (1% Bronopol), 72 (0.25, 0.5, 1% Bronopol) No induction (often prior contact with Bronopol by treatment with Bronopol-containing cream) epidermal challenge application	Due to the relatively high frequency of irritation reactions after treatment with 1% Bronopol, skin reactions at this dose level were considered not to be (clinically) relevant. Clinically relevant non-irritant positive skin reactions were seen in 9/72 (12.5%) patients after challenge application with 0.25% and/or 0.5% Occasionally sensitising in patients with skin lesions pre-treated regularly with Bronopol-containing cream (whole body application) sensitisation rate 12.5%	[redacted] 1983 (A6_01_5-4, A6.12.6_03)
Study with volunteers, **KEY STUDY**	0.5% Bronopol in petrolatum	Humans patients with (suspected) contact dermatitis in two series: 11443 and 1871 patients No induction epidermal challenge application	No. sensitised/total no.: 134/11443 and 32/1871 irritation also observed (lower incidence than sensitisation reactions). Sensitisation rates 1.1%-2.0% Irritation rates 0.8% - 0.4% Reaction index: 0.6 – 0.2Occasionally sensitising in patients with suspected contact dermatitis (potentially sensitive	[redacted] 1998 (A6_01_5-5, A6.12.6_08)

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)	Main effects, Observations	Reference
			subgroup)	
Study with volunteers, KEY STUDY	0.25%, 0.5% or 1% Bronopol	Humans patients in dermatology clinic three groups: 93 (prior exposure to Bronopol expected) and 2059 (with hand and/or face dermatitis) No induction (for one subgroup prior exposure to Bronopol) epidermal challenge application	No. sensitised/total no.: 2/93 (1% challenge, prior exposure to Bronopol suspected) 8/1996 (0.5% challenge) 1/63 (0.25% challenge) Irritation responses occurred at similar or higher incidences than allergic responses. Occasionally sensitising in potentially sensitive subgroup (patients in dermatology clinical; mostly with hand and/or face dermatitis) overall sensitisation rate 0.46%	[redacted] 1997 (A6_01_5-6, A6.12.6_09)
Study with volunteers, supportive	challenge probably with 0.5% Bronopol (the 5% stated in one Table may by a typing error)	Humans patients of 7 UK Patch test clinics (contact dermatitis suspected) in total 3062 (unclear whether all were treated with Bronopol) No induction	Occasionally sensitising in potentially sensitive subgroup (patients of Patch test clinics) Mean sensitisation rate 0.8% (0.5% irritation rate)	[redacted] 2003 (A6_01_5-7)
Study with volunteers, Supportive **Case report**	challenge with 0.25% Bronopol	Human patient with suspected adverse drug reactions 1 male	One patient with sensitising reactions to Bronopol (prior contact with Bronopol unknown)	[redacted] 1986 (A6_01_5-8)
Study with volunteers, supportive	challenge with 0.1% and 0.25% Bronopol	Humans patients of a Dermatology clinic 414	No. sensitised/total no.: 6/414 (for 5 of the positive	[redacted] 1987 (A6_01_5-9, A6.12.6_07_b)

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)	Main effects, Observations	Reference
		No induction	patients prior treatment of eczema with Bronopol containing cream) Occasionally sensitising in patients of dermatology clinic (mostly after probably long-term pre-treatment of skin lesions with Bronopol containing cream) sensitisation rate 1.4%	
Study with volunteers, supportive	challenge with 0.5% Bronopol	Humans patients of 7 European contact clinics totally 8149 No induction	No. sensitised/total no.: 38/8149 of which 17/8149 were considered of clinical relevance (prior contact with Bronopol) 10/8149 irritation reactions Occasionally sensitising in patients of contact clinics sensitisation rate (clinical relevance) 0.21%	[redacted] 1990 (A6_01_5-10, A6.12.6_07_a)
Review article on sensitisation to Biocides, supportive		The published sensitisation rates were often achieved in sensitive subgroups (patients of dermatology clinics – often with skin lesions), which are therefore not representative for the general population		[redacted] 2000 (A6_01_5-11)
Study with volunteers, Supportive	Induction concentration (5% Bronopol) Challenge performed with irritating concentration (2.5% Bronopol)	Humans healthy human volunteers 93 males 10 epidermal inductions in 3 weeks epidermal challenge 2 weeks after last induction application	5% induction concentration has a strong skin sensitization potential. No. sensitised/total no.: 11/93	[redacted] 1973 (A6_01_5-12)

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)	Main effects, Observations	Reference
			Result of 12% skin reactions were considered to be due to irritation according to a later publication by the same author ([redacted] 1977)	
Study with volunteers, Supportive	Induction with 2% Bronopol and Induction with 5% Bronopol Challenge performed with irritating concentration (2.5% Bronopol)	Humans healthy human volunteers 66, 93 males 10 epidermal inductions in 3 weeks epidermal challenge 2 weeks after last induction application	No. sensitised/total no.: 0/66 (induction with 2% Bronopol) 11/93 (induction with 5% Bronopol) Result of up to 12% skin reactions were considered to be due to irritation according to a later publication by the same author ([redacted] 1977)	[redacted] 1974 (A6_01_5-13)
Study with volunteers, Supportive **Case report**	challenge with 0.25% Bronopol	Human patient with skin rash 1 female No induction (prior occupational exposure to Bronopol)	One patient with sensitising reactions to Bronopol (prior occupational contact with Bronopol)	[redacted] 2000 (A6_01_5-14)
Review of sensitisation potential of several compounds, supportive		Humans evaluation of published data	Bronopol was put into the category 'reasonable indications for contact allergic effect'	[redacted] 2004 (A6_01_5-15)
Study with volunteers, supportive	0.25, 0.5 and 1 % Bronopol in pet.	Humans 7 patients (male and female) with dermatitis Patch testing	No. sensitised/total no.: 7/7 (6 at 0.25 % and one at 1 %)	[redacted] 1983 (A6.12.6_05)

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study (e.g. description of the test subjects (general population / selected / unselected dermatitis patients / workers), exposure data (induction dose, daily and overall exposure)	Main effects, Observations	Reference
Study with volunteers, supportive	1 % Bronopol in pet.	Humans 2298 patients (male and female) Patch testing over 2 years	No. sensitised/total no.: 20/2298 patients react to Bronopol Sensitisation rate 0.8 % Only a small number of subjects react to Bronopol. False positive results may be seen by approaching the irritancy threshold of Bronopol (0.5 – 1 %)	[redacted] 1986 (A6.12.6_06)

No other studies relevant for skin sensitisation are available.

A.3.5.1 Short summary and overall relevance of the provided information on skin sensitisation

Two guinea pig maximisation tests were conducted with Bronopol following the procedure of Magnusson and Kligman (OECD 406) ([redacted] 2001 (A6_01_5-1), [redacted] 1976 (A6.01.5_01)). The earlier maximisation test ([redacted] 1976; A6.01.5_01)was conducted according the acknowledged Magnusson and Kligman procedure which preceeded OECD 406with the exception that four challenges were performed instead of one and a cross-challenge with formaldehyde was included during the fourth challenge. After the first challenge no animals showed a positive skin reaction, whereas after the third and the fourth challenges a positive skin reaction was observed in 2/10 animals (20%). According to (EC) No 1272/2008 (CLP Regulation) section 3.4.2.2.3.1., when an adjuvant type test method for skin sensitisation is used, a response of at least 30 % of the animals is considered as positive to classify. The fourth challenge (0.2% bronopol) was conducted to see whether the sensitising potential of bronopol decreased with decreasing test concentration. The findings indicate that the sensitisation was not reduced at 0.2% challenge. Moreover, none of 9 animals were sensitised after cross-challenge with formaldehyde. Therefore, due to the low rate of positive skin reactions, Bronopol was considered not skin sensitising in the test. Furthermore, Bronopol-mediated sensitisation against formaldehyde was ruled out as well. The negative result in the GPMT was confirmed in the GLP compliant study according to OECD TG 406 performed by [redacted] (2001; A6_01_5-1), in which where no skin sensitising effects were observed in the maximisation test as well. In addition, Bronopol was not sensitising in an GLP-compliant LLNA test according to OECD 429 ([redacted] 2005; A6_01_5-2). In this test, 6 female mice were treated with an application of 25 µL/ear on the dorsal surface of both ears at 0% (vehicle), 0.4%, 2%, 10% or 50% Bronopol in DMSO on three consecutive days (due to severe irritation reactions at 50% Bronopol in DMSO, these animals were removed from the test). On day 6, mice received a 250 µL i.v. injection of 20 µCi ^{3}H-thymidine in phosphate-buffered saline.

About 5 hours after application of ^{3}H-thymidine, mice were sacrificed and a single cell suspension of the auricular lymph nodes from one mouse was prepared. Topical application of 0.4%, 2% or 10% Bronopol elicited Stimulation Indexes (SI) that were respectively, 1.6-, 2.4-, and 2.4- fold greater than vehicle controls. These SI were calculated using the absolute disintegrations per minute (dpm) value for each mouse as the numerator and the mean dpm value from the vehicle control mice as the denominator. Therefore, according to CLP Regulation (section 3.4.2.2.3.1.), Bronopol did not show dermal sensitization potential in the mouse LLNA as the lymph nodes draining the area of topical application did not demonstrate a 3-fold proliferation (SI) when compared to vehicle – treated mice.

Thus, based on the available animal studies Bronopol is not considered to be skin sensitising according to CLP Regulation

In addition, there are several examples in the published literature showing that bronopol induces skin sensitisation in humans. Generally, human testing was normally done in patients of dermatology clinics where skin lesions (e.g. dermatitis) or an allergic dermatitis was suspected. The sensitisation rates were ranging from 1.1% up to 12.5% when specifically, sensitive subgroups of patients were tested.

In healthy volunteers with unknown pre-exposure, Bronopol showed no skin sensitising activity ([redacted] 1977; A6_01_5-3, A6.12.6_02). The study protocol consisted of a three-week induction period with 5% Bronopol, an irritant concentration, followed by a two-week incubation period and an elicitation with Bronopol at a sub-irritant concentration (0.25 %). Skin irritation appeared after induction with 5% bronopol. However, no indication of skin sensitization potential can be established. The authors concluded that in previous studies false positive, irritant dermal responses were seen due to the high concentration of Bronopol (2.5%) tested ([redacted], 1973 (A6_01_5-12), [redacted], 1974 (A6_01_5-13)). In subsequent studies this concentration was recognized as irritant per se, and thus unsuitable for use in challenge application in such type of study.

Some reliable human patch test data in dermatitis patients are also available. In a test in human ([redacted], 1998; A6_01_5-5, A6.12.6_08), an epidermal challenge of 0.5% bronopol was applied to patients where allergic contact dermatitis was suspected. These exposures related observations in humans demonstrate that dermal exposure of patients to the test substance Bronopol results in signs of irritation and positive skin reactions. It has to be pointed out that [redacted] (1998; A6_01_5-5, A6.12.6_08) investigated the patch test reaction to Bronopol (0.5 % in petrolatum) in 11443 patients (preservative series, PS) and 1871 patients (industrial biocides, IB). The reaction index (ratio between irritant reactions and sensitising reaction) was 0.6 and 0.2, respectively. 134 cases (1.2 %) in preservative series and 32 cases (1.8 %) in industrial series showed positive reactions. In the PS group, the age-adjusted standardized sensitisation rate for men and women were 1.3% and 1.1%, respectively. In the IB group, an age-adjusted standardized sensitisation rate of 2.0 was seen for men. Moreover, the sensitization rate was higher in patients in the age group older than 40, than in younger patients. In patients with allergic skin dermatitis, the sensitization rates were from 1.1% to 2.0%. However, concerning these sensitisation rates, the examined subgroup of patients (where allergic dermatitis was suspected) was considered to be not representative of the general population. Other studies in dermatitis patients also found a rather low incidence of positive patch test reactions to Bronopol in this sensitive population ([redacted] 1990 (A6_01_5-10, A6.12.6_07_a): 0.21% (clinically-relevant reactions), i.e. 17 of 8149 patients; [redacted] 1987 (A6_01_5-9, A6.12.6_07_b): 1.4%, i.e. 6 of 414 patients; [redacted] 1997 (A6_01_5-6, A6.12.6_09): 0.46%, i.e. 8 of 1996 patients). Furthermore, [redacted] (1986; A6.12.6_06) postulated that positive results in patch testing (sensitisation rate 0.8%) may be seen by approaching the irritancy threshold of Bronopol (0.5-1 %).

In general, Bronopol is widely used as a preservative in cosmetics and toiletries. Allergic contact dermatitis to Bronopol has been reported, in a patch test with Bronopol in different concentrations (0.25 %, 0.5 %, 1 % Bronopol) ([redacted], 1983; A6.12.6_05). 57 humans were tested with 1% bronopol and 72 patients were tested with 0.25%, 0.5% and 1% bronopol. The highest concentration (1%) produced irritant reactions in 23/129 patients (18%). After the application of 0.25% and 0.5% bronopol, 12/72 patients had non-irritant positive reactions from which 9/72 patients showed clinically relevant reactions. Therefore, there is a sensitisation rate of 12.5%. Regarding this sensitisation rate, it should be noted that the group of patients tested was not representative for the general population but can be considered as an especially sensitive subgroup (patients already had dermatitis prior to contact with Bronopol potentially leading to an increased absorption of Bronopol) and had a high frequency of Bronopol exposure (routine, whole-body treatment with a cream containing 0.05% Bronopol as a preservative prior to the Bronopol challenge application). Furthermore, the patients with positive skin reactions also revealed positive reactions to other antigens (including formaldehyde).

Allergic reactions were observed after dermal exposure to Bronopol in patients, too ([redacted], 1997; A6_01_5-6, A6.12.6_09). A patch test was performed in humans ([redacted], 1997; A6_01_5-6, A6.12.6_09), who had been previously exposed to Bronopol. 63 patients who were expected to have been previously exposed were given a dose of 0.25% bronopol, 1996 patients were administered a dose of 0.5% Bronopol and 93 received 1% bronopol. The evaluations were carried out 1 hour and 96 hours after the challenge. The allergic skin responses were 1/63, 8/1996 and 2/93. The irritation responses were 1/63, 11/1996 and 4/93. Therefore, Bronopol had a low (0.46%) sensitisation rate after application at a concentration of 0.5% to patients, which had hand and/or face dermatitis. Bronopol as broad spectrum antimicrobial was assessed using the DRAIZE procedure on normal human test subjects ([redacted] and [redacted], 1973). Human test results showed that at 5 % induction concentration it has a strong skin sensitisation potential (No. sensitised/total no:11/93; 12% skin reactions). However, these skin reactions were considered to be due to irritation according to a later publication by the same author detailed above ([redacted] 1977; A6_01_5-3, A6.12.6_02). Under alkaline conditions, Bronopol liberates formaldehyde. In some subjects who were sensitive to Bronopol, skin reactions were elicited when the subjects were tested with formalin. This suggests that there were other antigenic determinants in the test substance in addition to formaldehyde ([redacted] and [redacted], 1973, Antimicrobials: Experimental contact sensitisation in man, J. Soc. Cosmet. Chem. 24, 399-421). Patients with dermatitis showed a positive skin reaction to Bronopol at 1% ([redacted], 1997 (A6_01_5-6, A6.12.6_09) see A.3.3 Skin corrosion and irritation) or at 0.5% and 0.25% ([redacted], 1983).

Human data from clinical epidemiological studies showed ambiguous evidence that Bronopol has sensitising properties. Some studies showed positive reactions in human patch tests, which in some cases might be and in other cases are clearly false-positive due to irritant properties of Bronopol. The overall incidence of positive reactions was very low (approx. 1%) and the degree of severity in the skin reactions was low. In several other studies even no dermal reactions were observed. The preexposure towards Bronopol of the individuals tested and the purity of Bronopol solutions used for patch test analysis often were not documented. Additionally, the concentrations used for induction and/or elicitation were often very high, i.e. irritating, thus the difference between irritant and sensitising reactions were difficult to distinguish.

Furthermore, the CLP guidance considers data from animal studies as more reliable that human clinical data (3.4.2.2.4.2.: "Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with

volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on skin sensitisation are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken. [...]"), because the documentation and the testing is much more defined. In OECD guideline-conform standard animal studies, Bronopol showed no sensitising potential.

Therefore, in a weight of evidence approach it can be concluded that Bronopol might have some weak sensitising properties, but that this evidence is not sufficient for classification according to the above mentioned CLP Regulation. In addition, due to the irritant properties of Bronopol, exposure to Bronopol is sufficiently excluded by risk management measures in the respective uses and therefore the risk of potential sensitisation can be sufficiently controlled.

A.3.5.2 Comparison with the CLP criteria

Bronopol was found negative in two GPMTs (OECD TG 406) and an LLNA test (OECD TG 429). The earlier maximisation test ([redacted] 1976; A6.01.5_01) showed a positive skin reaction in 2/10 animals (20%) which is lower than the 30% value according to (EC) No 1272/2008 (CLP Regulation) section 3.4.2.2.3.1. considered for classification. This negative result was confirmed in another GPMT study ([redacted] 2001; A6_01_5-1) in which no skin sensitising effects were observed. Furthermore, as the stimulation index in an LLNA test was <3, Bronopol did not show dermal sensitization potential according to CLP Regulation (section 3.4.2.2.3.1.),

In healthy volunteers, Bronopol did not induce skin sensitisation, while low incidences of dermal allergic responses were founded in contact dermatitis patients.

Thus, classification and labelling for skin sensitisation according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.5.3 Conclusion on classification and labelling for skin sensitisation

Based on the available animal and human data, no classification and labelling for skin sensitisation according to CLP criteria should be warranted under Regulation (EC) No 1272/2008.

A.3.5.4 Overall conclusion on skin sensitisation related to risk assessment

Not applicable for the CLH report.

A.3.6 Respiratory sensitisation

No data on respiratory sensitisation is available.

Data waiving	
Information requirement	Respiratory sensitisation (Annex II, Title 1, 8.4)
Justification	It is stated in the Guidance on the BPR Vol. III, Part A, v.1.2, that "there are currently no standard tests and no OECD test guidelines available for respiratory sensitisation. Since an active substance identified as a skin sensitiser can potentially induce a hypersensitivity reaction, potential respiratory sensitisation and respiratory elicitation after dermal sensitisation should be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects." As there are currently no appropriate tests available and there is no indication of respiratory sensitisation effects for Bronopol, respiratory sensitisation cannot be opened for consultation.

A.3.7 Repeated dose toxicity / STOT RE

A.3.7.1 Short term repeated dose toxicity

A.3.7.1.1 Short-term oral toxicity

Table A-27: Summary table of oral short-term animal studies (usually 28-day studies)

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key / supportive study	**Species, Strain, Sex, No / Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including also target organs)**	**Remarks (e.g. major deviations)**	**Reference**
Up to 14 days palatability study, oral (via drinking water) **Rel. 2** **Supportive**	Dog Beagle 3 females/ group	Bronopol Dose levels: 0, 0.005, 0.05, 0.1% and 0.5% (mg/kg bw dose not determined) Treatment: Daily via drinking water	NOAEL = 0.05% based on severe clinical signs at 0.1%	Low and medium low dose: no effects on clinical signs/water consumption medium high and high dose: severe clinical signs (emesis, loose stool with blood, vomit with blood poor general appearance)		[redacted] 2006 A6_03_1-01

OECD 407 **28 days, oral (via drinking water)** **GLP** **Rel. 1** **Supportive**	Dog Beagle 2 sex/group	Bronopol (99.9%) Dose levels: 0, 0.005, 0.025, 0.05% equivalent to 0, 4.47, 20.7, 40.6 mg/kg bw/day (males) and 0, 4.27, 15.4, 32.7 mg/kg bw/day (females) Treatment: Daily via drinking water for 28 days	NOAEL (local) = 0.025% (20.7/15.4 mg/kg bw/day for males/females) based on mild irritation in stomach at 0.05% NOAEL (systemic) = 0.05% (40.6/32.7 mg/kg bw/day for males/females)	low dose: very slight, multifocal, subacute to chronic inflammation of the nasal mucosa medium dose: as low dose + very slight multifocal hypertrophy of mucous cells in stomach (1 dog) high dose: as low dose + very slight multifocal hypertrophy of mucous cells in stomach (3 dogs)		[redacted] 2006 A6_03_1-2

A sub-acute oral toxicity test was performed in Beagle dog ([redacted] 2006; A6_03_1-2) under guidelines. Three doses plus a control were tested during 28 days; for males 4.47, 20.73 and 40.59 mg/kg bw/day and for females 4.27, 15.40 and 32.65 mg/kg bw/day in drinking water. No indications for systemic toxicity were seen. However, irritation of nasal mucosa was seen at all doses. Multifocal hypertrophy of mucous cells in stomach was observed in three dogs at the high dose. The NOAEL for local effects was 20.7 and 15.4 mg/kg bw/day for males and females, respectively on the basis of local irritation in stomach at the higher dose level. The NOAEL for systemic toxicity was 40.6 and 32.7 mg/kg bw/day for males and females, respectively.

No human data on short-term oral toxicity is available.

A.3.7.1.2 Short-term dermal toxicity

Table A-28: Summary table of dermal short-term animal studies (usually 28-day studies)

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Surface area, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including target organs)**	**Remarks (e.g. major deviations)**	**Reference**
Non-guideline **21 days**	Rabbit, New Zealand White,	Bronopol (purity not stated)	NOAEL (local) = 0.2% Bronopol	No mortality and no signs of systemic		[redacted] 1973 A6.03.2_01

Non-GLP **Rel. 2** **Key Study**	male and female 5/sex/group	<u>Concentrations</u>: 0.2% and 0.5% in 2.5% methylcellulose (1 ml/kg bw) <u>Treatment</u>: Area: 10 cm^2 6 h/day, 7 d/week, non-occlusive <u>Remark</u>: Removal of residual test substance after each treatment period by washing with a warm dilute soap solution and rinsing with clean water. Skin was blotted dry with absorbent paper.	(2 mg/kg/day) based on skin irritation at 0.5% NOAEL (systemic) = 0.5% Bronopol (5 mg/kg/day)	toxicity. <u>Skin irritation</u>: <u>Vehicle control (2.5% methylcellulose):</u> Slight to well-defined erythema. <u>0.2% Bronopol</u>: Findings similar control group. <u>0.5% Bronopol</u>: Moderate erythema and edema, with extensive scabbing at the application site. Scabbing was considered to result from the healing process caused by the moderate cutaneous irritation described above.		

Repeated dermal application of bronopol of unknown purity ([redacted] 1973; A6.3.2_01) to male and female rabbits for 3 weeks (6 hours/day, 7 days/week) at concentrations of 0.2 and 0.5% (2 mg/kg bw/day and 5 mg/kg bw/day) in methylcellulose suspension was not associated with signs of systemic toxicity or deaths. Severe skin irritation was observed after application of 5 mg bronopol/kg bw/day, whereas 2 mg bronopol/kg bw/day caused skin reactions comparable to those elicited by the vehicle alone. Skin findings related to 5 mg bronopol/kg bw/day included erythema and edema with extensive scabbing at the application site. Thus, on the basis of skin irritation the NOAEL was 2 mg/kg bw/day due to the irritations at the higher dose.

No human data on short-term dermal toxicity is available.

A.3.7.1.3 Short-term inhalation toxicity

No data on short-term inhalation toxicity is available.

Data waiving	
Information requirement	Short-term repeated dose toxicity (inhalation) (Annex II, Title 1, 8.9.1)
Justification	A short-term repeated dose toxicity study by inhalation route of exposure does not need to be conducted since reliable short-term (28-day) toxicity studies by oral and dermal route ([redacted]

	2006 (A6_03_1-2) and [redacted] 1973 (A6.03.2_01)), as well as sub-chronic oral toxicity studies ([redacted] 2001 (A6_04_1-1), [redacted] 2006 (A6_04_1-2), [redacted] 2007 (A6_04_1-3), [redacted] 1973 (A6.04.1_01), and [redacted] 1973 (A6.04.1_02)) and chronic toxicity studies are available by oral ([redacted] 1976 (A6.07_01_a), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] 1986 (A6.07_01_e), and [redacted] 1985 (A6.07_01_f)) and dermal ([redacted] 1975 (A6.07_02_a), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), and [redacted] 1973 (A6.07_02_e)) routes of exposure. In accordance with Regulation (EU) No 528/2012 (section 8.9), only one route of administration is necessary, and the oral route is the preferred one. Owing to the expected use patterns of Bronopol as biocide and the physico-chemical properties of Bronopol repeated inhalation exposure of consumer and worker to the substance is unlikely. Furthermore, systemic reference values derived from studies with oral exposure to Bronopol do also cover the inhalation route.

A.3.7.1.4 Overall conclusion on short-term repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.2 Sub-chronic repeated dose toxicity

A.3.7.2.1 Sub-chronic oral toxicity

Table A-29: Summary table of oral sub-chronic animal studies (usually 90-day studies)

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including also target organs)**	**Remarks (e.g. major deviations)**	**Reference**
OECD 408 90 days, oral (via drinking water) GLP Rel. 1 Supportive	Rat Wistar 10 sex/group for recovery 10/sex for control and high dose	Bronopol (98.7%) <u>Dose levels</u>: 0, 60, 250, 1000 ppm, equivalent to 0, 6.2, 24.3, 83.9 mg/kg bw/day	NOAEL = 24.3/25.5 mg/kg bw/day for males/females LOAEL = 83.9/86.0 mg/kg	<u>low dose</u>: no effects <u>medium dose</u>: no adverse effects (↓ water consumption, males; ↑ kidney weight, females)		[redacted] 2001 A6_04_1-1

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
		(males) and 0, 6.8, 25.5 86.0 mg/kg bw/day (females) Treatment: daily in drinking water	bw/day for males/ females	high dose: ↓ Hb and MCVC (males), ↓ water consumption, ↓ urine volume and ↑ osmolarity; ↑ O-DEM activity (female liver); kidney: ↑ weight, basophilic tubules and hyaline casts; all effects reversible after recovery except hyaline casts (loops of Henle)		
OECD 408 **90 days, oral (via drinking water)** **GLP** **Rel. 2** **Supportive**	Rat Sprague-Dawley 8 sex/group	Bronopol (99.9%) Dose levels: 0, 0.025, 0.075, 0.15%, equivalent to 0, 21.8, 59.2, 124.7 mg/kg bw/day (males) and 0, 28.2, 78.2, 136.3 mg/kg bw/day (females) Treatment: daily in drinking water	NOAEL = 59.2 (0.075%)/136.3 (0.15%) mg/kg bw/day for males/females LOAEL = 124.7 (0.15%) mg/kg bw/day for males In the absence of adverse effects no LOAEL could be determined for females	low and medium dose: no adverse effect (slightly ↓ water consumption) high dose: ↓ bw development, food and water consumption; kidney (increased weight, nephropathy)		[redacted] 2006 A6_04_1-2
OECD 409 **90 days, oral (via**	Dog Beagle	Bronopol (99.9%)	NOAEL = 0.05% mg/kg bw/day	Low, medium and high dose: no		[redacted] 2007 A6_04_1-3

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
drinking water) **GLP** **Rel. 2** **Supportive**	4 sex/group	Dose levels: 0%, 0.005%, 0.025%, 0.05%, equivalent to 3.76, 15.0, 28.4 mg/kg bw/day (males) and 3.76, 18.8, 32.2 mg/kg bw/day (females) Treatment: daily in drinking water	for males/females LOAEL > 0.05% mg/kg bw/day for males/females	treatment-related adverse effects		
Similar to OECD 408 **13 weeks** **Non-GLP** **Rel. 2** **Supportive**	Rat, CD, male and female 20 animals/sex/group	Bronopol, (98–102%) Dose levels: 20, 80, 160 mg/kg bw/day (aqueous sol.) Treatment: Oral, gavage, 7 days/week Remark: In the 160 mg/kg group, 4 males and 5 females died following first dosage. These rats were replaced by rats of equivalent	NOAEL < 20 mg/kg bw/day LOAEL = 20 mg/kg bw/day	Mortality: Treatment related mortality for both sexes in mid and high dose group. In the low dose group, one female died, however, not treatment-related as revealed by necropsy. Clinical symptoms: Respiratory distress and abdominal distention were seen in all groups. In the low dose group, 1 male suffered from transient		[redacted] 1973 A6.04.1_01

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
		weight taken from among spare rats, which had been treated with the appropriate dosage.		respiratory distress and recovered after 2 weeks. Respiratory distress at 80 mg/kg bw was less severe than at 160 mg/kg bw, with gradual recovery. Body weight, food consumption & conversion efficiency: Body weight gain, food consumption and food conversion efficiency in the low dose group was within the range of the control group. In the mid and high dose groups all parameters affected by treatment during the first week, thereafter recovery was seen in the mid dose group. Ophthalmology: Inconspicuous. Haemaotology, clinical chemistry,		

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
				urinalysis: Inconspicuous. Necropsy: Few organs with significant changes in absolute and/or relative weights, which in part were related to changes in the body weight (at low and mid dose). No gross pathological treatment-related abnormalities. Histopathology revealed some renal tubular abnormalities in few animals (males, 20 & 80 mg/kg bw); which were not evident in control and were therefore seen as treatment-related. However, no dose-dependency could be established.		
Similar to OECD 409 13 weeks Non-GLP	Dog, Beagle, male and female, 3 animals/sex/group	Bronopol (99.2%) Dose levels: 4, 8 and 20 mg/kg	NOAEL = 8 mg/kg bw/day LOAEL = 20	Mortality: None Clinical symptoms: Vomiting observed in all treatment		[redacted] 1973 A6.04.1_02

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
Rel. 2 **Key**		bw/day (aqueous sol.) Treatment: Oral, gavage, 7 days/week Remark: 20 mg/kg bw/day was chosen as highest test dose on the basis of results of a range-finding study.	mg/kg bw/day	groups during the first 6 weeks of the study stopped after changing the dosing/feeding routine after week 6, except for one dog in the high dose group. Body weight gain, food and water consumptions, ophthalmological findings: Within the range of the control group for all treatment groups. Haematology, clinical chemistry, and urinalysis: After 6 weeks of treatment, blood pigments and red blood cells were found in the urine of two and one female(s) of the low and high dose group, respectively. After 12 weeks of treatment, statistically significant decrease		

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including also target organs)	Remarks (e.g. major deviations)	Reference
				in mean total white cell counts was observed in the mid and high dose group when compared to control; however, the findings remained within normal limits. Necropsy: In the male high dose group increased relative liver weight and increased absolute and relative spleen weights were observed. No related gross pathological and histopathological changes were reported.		

Bronopol was given orally to groups of 20 male and 20 female CD rats at concentrations of 0, 20, 80 or 160 mg/kg bw/day during 90 days ([redacted] 1973; A6.04.1_01). Mortality occurred in 49 animals at the highest dose (including replacement animals) and 16 animals died at the dosage of 80 mg/kg bw day. Histopathology showed renal changes (distended renal tubules containing eosinophilic material and mononuclear cell infiltration in the adjacent interstitial tissue) at the medium and low dose (80 and 20 mg/kg bw day). Therefore, no NOAEL was established from this study as the low dose of 20 mg/kg bw/day showed clear evidences of renal damages.

A 90-day oral study in rats ([redacted] 2001; A6_04_1-1) was carried out under guidelines. Rats were given by drinking water 6.2, 24.3 or 83.9 mg/kg bw/day (males) and 6.8, 25.5 and 86.0 mg/kg bw/day (females). The observation period was 4 weeks. At the low-dose

level no effects were seen. At the high-dose level slight reduction of body weight development and food consumption was observed. Histopathological changes in the kidney (increase of the incidence of hyaline casts in the loops of Henle) were also observed in the highest dose group. In addition, some reduced red blood cell parameters (Hb and MCVC) or a slight increase in O-DEM activity in the liver was noted. The NOAEL for this subchronic oral study was 24.3 and 25.5 mg/kg bw/day for males and females, respectively, under the basis of the histopathological changes observed in the kidney.

A second oral subchronic study was performed in rats under guidelines ([redacted] 2006; A6_04_1-2). During 90 days, rats were orally administered with 21.82, 59.16 or 124.71 mg/kg bw/day for males and 28.18, 78.19 and 136.31 mg/kb bw/day for females in drinking water. The kidney was identified as a target organ in this study. In the mid-dose level, a non-significant decrease in the water consumption was observed. A decrease in the body weight development and a reduction in the food and water consumption were seen at the high-dose level in males. An increased kidney weight and nephropathy (tubular epithelial degeneration with regeneration, dilatation of medullary tubules and interstitial inflammation) were also observed at the high-dose level in males. The male NOAEL was 59.16 mg/kg bw/day under the basis of nephropathy, an increase in the kidney weight, and a reduction in the body weight development, food and water consumption. However, a female NOAEL cannot be established due to the absence of adverse effects.

In a 90-day drinking water study in dogs ([redacted] 2007; A6_04_1-3) under guidelines, 4 male and 4 female Beagle dogs for each dose were administered orally by drinking water with 3.76, 15.04 or 28.29 mg/kg bw/day for males and 3.76, 18.75 or 32.16 mg/kg bw/day. No clinical signs, organ weight changes or systemic toxicity were reported. The histopathology and the macroscopical and ophthalmological analysis did not find any changes. A trend towards reduced overall water consumption was noted at the high dose level but it was considered to be of not toxicological relevance and was not statistically significant. Therefore, a NOAEL could not be established due to the absence of adverse effects.

Groups of 3 male and 3 female Beagle dogs ([redacted] 1973; A6.04.1_02) were given 0, 4, 8 or 20 mg/kg bw/day in distilled water by gavage for a period of 13 weeks during 7 days/week. No cases of mortality were reported at any of the tested doses. However, clinical signs were observed, like increased liver weight of the 20 mg/kg bw group. Also, the mean absolute and relative spleen weights for the 20 mg/kg bw group were significantly above the control value. No related gross pathological and histopathological changes were reported. The NOAEL was set at 8 mg/kg bw day under the bais of increase in liver and spleen weight.

No human data on sub-chronic oral toxicity is available.

A.3.7.2.2 Sub-chronic dermal toxicity

No data on sub-chronic dermal toxicity is available.

Data waiving	
Information requirement	Sub-chronic repeated dose toxicity (dermal) (Annex II, Title 1, 8.9.2)
Justification	A sub-chronic repeated dose toxicity study by dermal route of exposure does not need to be conducted since reliable short-term (28-day) toxicity studies by oral and dermal route ([redacted] 2006 (A6_03_1-2) and [redacted] 1973 (A6.03.2_01)), as well as sub-chronic oral toxicity studies ([redacted] 2001 (A6_04_1-1), [redacted] 2006 (A6_04_1-2), [redacted] 2007 (A6_04_1-3), [redacted]

	1973 (A6.04.1_01), and [redacted] 1973 (A6.04.1_02)) and chronic toxicity studies are available by oral ([redacted] 1976 (A6.07_01_a), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] 1986 (A6.07_01_e), and [redacted] 1985 (A6.07_01_f)) and dermal ([redacted] 1975 (A6.07_02_a), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), and [redacted] 1973 (A6.07_02_e)) routes of exposure.. In accordance with Regulation (EU) No 528/2012 (section 8.9), only one route of administration is necessary, and the oral route is the preferred one. Moreover, based on the acute toxicity studies, dermal toxicity is lower than oral toxicity, as is dermal compared to oral absorption and there is no substance known to be of dermal toxicity structurally related to Bronopol. Furthermore, systemic reference values derived from studies with oral exposure to Bronopol do also cover the dermal route.

A.3.7.2.3 Sub-chronic inhalation toxicity

No data on sub-chronic inhalation toxicity is available.

Data waiving	
Information requirement	Sub-chronic repeated dose toxicity (inhalation) (Annex II, Title 1, 8.9.2)
Justification	A sub-chronic repeated dose toxicity study by inhalation route of exposure does not need to be conducted since reliable short-term (28-day) toxicity studies by oral and dermal route ([redacted] 2006 (A6_03_1-2) and [redacted] 1973 (A6.03.2_01)), as well as sub-chronic oral toxicity studies ([redacted] 2001 (A6_04_1-1), [redacted] 2006 (A6_04_1-2), [redacted] 2007 (A6_04_1-3), [redacted] 1973 (A6.04.1_01), and [redacted] 1973 (A6.04.1_02)) and chronic toxicity studies are available by oral ([redacted] 1976 (A6.07_01_a), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] 1986 (A6.07_01_e), and [redacted] 1985 (A6.07_01_f)) and dermal ([redacted] 1975 (A6.07_02_a), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), and [redacted] 1973 (A6.07_02_e)) routes of exposure. In accordance with Regulation (EU) No 528/2012 (section 8.9), only one route of administration is necessary, and the oral route is the preferred one. Owing to the expected use patterns of Bronopol as biocide and the physico-chemical properties of Bronopol repeated inhalation exposure of consumer and worker to the substance is unlikely. Furthermore, systemic reference values derived from studies with oral exposure to Bronopol do also cover the inhalation route.

A.3.7.2.4 Overall conclusion on sub-chronic repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.3 Long-term repeated dose toxicity

A.3.7.3.1 Long-term oral toxicity

Table A-30: Summary table of oral long-term animal studies

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including target organs)	Remarks (e.g. major deviations)	Reference
Non-guideline 104 weeks (oral via drinking water) Non-GLP Rel. 2 Key	Rat, Sprague-Dawley, male and female, Main test: 45/sex/group Satellite group for blood sampling/clinical pathology: 15/sex/group	Bronopol (purity not stated) Dose levels nominal: 10, 40, 160 mg/kg bw/day Re-evaluated doses: 7, 32, 142 mg/kg bw/day Treatment: oral, drinking water	NOAEL = 10 mg/kg bw/day LOAEL = 40 mg/kg bw/day	Systemic toxicity: Treatment- and dose-related effects seen at 40 and 160 mg/kg bw/day (reduction in food consumption and body weight gain; additional findings in the high dose group: increased mortality, reduction in grooming activity) Effects due to palatability: In all treatment groups reduced water uptake was observed, resulting in decreased urine production and further associated with exacerbated incidence of glomerulonephrosis,		A6.07_01_a(reported in [redacted] 1976 and US EPA RED 1995), [redacted] 1993 A6.07_01_b, [redacted] 1985 A6.07_01_c, [redacted] 1998 A6.07_01_d, [redacted] 1986 A6.07_01_e, [redacted] 1985 A6.07_01_f

Method, Duration of study, Route of exposure (gavage, in diet, other), Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including target organs)	Remarks (e.g. major deviations)	Reference
				which is regarded as a spontaneous occurring lesion in the used rat strain. Pathological findings, non-neoplastic findings: Lesions in the stomach and the gastric lymphnodes (all doses), attributed to the irritant potential of Bronopol. Incidences of squamous metaplasia in the salivary glands (often with inflammation and atrophic acini), which is a spontaneous occurring change in the used rat strain		

In a no guideline study ([redacted] 1976, [redacted] 1993, [redacted] 1985, [redacted] 1998, [redacted] 1986, and [redacted] 1985; A6.07_01_a to f), 45 male and 45 female Sprague-Dawley rats/group were exposed orally during 104 weeks to bronopol in acidified drinking water at concentrations designed to provide 0, 10, 40 or 160 mg/kg bw/day. Reevaluation of the provided doses considering the stability of bronopol suggested that that actual doses were 0, 7, 32, and 142 mg/kg bw/day. At the end of the exposure period all animals were examined for systemic toxicity and neoplasic changes. Mortality was significantly increased in the high dose group. Body weight gain and food

consumption were significantly reduced in the mid- and high-dose groups. Reduced water uptake resulting in decreased urine production and spontaneous glomerulonephrosis were considered to be caused by the reduced palatability of the drinking water. Lesions in the stomach included thickening of the non-glandular region were reported for the high dose group and were attributed to the irritant potential of the test substance. A treatment-related dilatation of the sinusoids in the gastric lymph nodes was reported for males and females of the high-dose group.

The most frequently observed tumours in this study were pituritary adenoma in male and female rats and mammary fibroadenoma in the females. For both tumours the incidences lacked a dose-response relationship. Neoplasic changes identified by histopathological examination of the stomach of high-dose rats that died during the exposure period were squamous cell papilloma associated with epithelial hyperplasia and ulceration. A more recent re-evaluation of the histopathological findings confirmed that these findings and papillomas were also reported for the forestomachs of the rats treated with bronopol. However, the fact that these findings mainly were seen in the high-dose group and were associated with ulceration is taken as indication they were a consequence of the irritant potential of bronopol rather than reflectiong a carcinogenic potential of the test substance. Thus, no carcinogenic potential could be evidenced for bronopol when applied orally under the test conditions chosen. Thus, the NOAEL of this study was set at 7 mg bronopol/kg bw/ day, on the basis of abnormalities of the salivary glands and reduction of body weight gain and food consumption, observed the next higher doses.

No human data on chronic oral toxicity is available.

A.3.7.3.2 Long-term dermal toxicity

Table A-31: Summary table of dermal long-term animal studies

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Surface area, Duration of exposure**	**NOAEL, LOAEL**	**Results (all dose levels including severity and magnitude of all effects, including target organs)**	**Remarks (e.g. major deviations)**	**Reference**
Non-guideline 80 weeks (dermal) Non-GLP Rel. 2 Key	Mouse CFLP mice Males and females 52/sex/dose	Bronopol (98 - 102%) Vehicle: 90% acetone in water Dose levels: 0, 0.2%, 0.5% corresponding to 0, 20, 50 mg/kg bw/day (assuming a body weight of 30 g/mouse)	NOAEL = 0.2% (20 mg/kg bw/day)	A slight but statistically significant decrease in mean body weight gain was reported for the males treated with 0.5% Bronopol for the period ranging from week 26 to week 52. Some mice of the group treated with 0.5% Bronopol showed slight loss in		A6.07_02_a (reported in [redacted] 1975 and US EPA RED 1995), [redacted] 1986 A6.07_02_b, [redacted] 1992 A6.07_02_c, [redacted] 1998 A6.07_02_d, [redacted] 1973 A6.07_02_e

Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Surface area, Duration of exposure	NOAEL, LOAEL	Results (all dose levels including severity and magnitude of all effects, including target organs)	Remarks (e.g. major deviations)	Reference
		Treatment: one application/day, 3 days/week (i.e. on Monday, Wednesday and Friday) for 80 weeks		hair around the treated skin area of mice during the first 3 weeks of treatment. No further treatment-related effects were seen. Mortality for the treated females (both test groups) was within control range and therefore inconspicuous (after 80 days: 35% mortality in the 0.2% test group and 40% mortality in the 0.5% group, versus 46% mortality in the control group). During gross pathology and histopathology, all reported findings were incidental and without relationship to the treatment with Bronopol.		

In an 80-week chronic toxicity and carcinogenicity study ([redacted] 1975, [redacted] 1986, [redacted] 1992, [redacted] 1998, and [redacted] 1973; A6.07_02_a to e), groups of 52 CFLP mice of each sex were given by dermal administration bronopol at a concentration of 20 and 50 mg/kg bw/day, 3 times/week during 80 weeks.

The assessment of the carcinogenicity of dermally applied bronopol provided information on systemic toxicity and non-neoplastic changes in CFLP mice. Mortality in both test groups was slightly increased for the males compared to control (50% mortality at 20 mg/kg bw/day

and 48% at 50 mg/kg bw/day vs. 36% in control after 80 weeks). However, the causes of death of the males were common for the strain used and the age of the animals and no relationship to treatment was evident. The repeated treatment with 50 mg/kg bw/day resulted in a slight but statistically significant decrease in mean body weight gain of males from week 26 to 52; slight hair loss around the treated skin area was seen during the first 3 weeks of treatment. Animals of the 20 mg/kg bw/day test group were inconspicuous compared to control.

No treatment-related systemic toxicity was observed after gross pathological and histopathological examination of animals that died during the experiment and those that were sacrificed at test ending.

The NOAEL was 20 mg/kg bw/day based in the observation of reversible skin tumours with no statistical significance at the 50 mg/kg bw/day dose.

No human data on chronic dermal toxicity is available.

A.3.7.3.3 Long-term inhalation toxicity

No data on long-term inhalation toxicity is available.

Data waiving	
Information requirement	Long-term repeated dose toxicity study (inhalation) (Annex II, Title 1, 8.9.3)
Justification	A long-term repeated dose toxicity study by inhalation route of exposure does not need to be conducted since reliable chronic toxicity studies are available by oral ([redacted] 1976 (A6.07_01_a), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] 1986 (A6.07_01_e), and [redacted] 1985 (A6.07_01_f)) and dermal ([redacted] 1975 (A6.07_02_a), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), and [redacted] 1973 (A6.07_02_e)) routes of exposure. In accordance with Regulation (EU) No 528/2012 (section 8.9), only one route of administration is necessary, and the oral route is preferred one. Owing to the expected use patterns of Bronopol as biocide and the physico-chemical properties of Bronopol repeated inhalation exposure of consumer and worker to the substance is unlikely. Furthermore, systemic reference values derived from studies with oral exposure to Bronopol do also cover the inhalation route.

A.3.7.3.4 Overall conclusion on long-term repeated dose toxicity related risk assessment

Not applicable for the CLH report.

A.3.7.4 Specific target organ toxicity – repeated exposure (STOT RE)

A.3.7.4.1 Short summary and overall relevance of the provided information on STOT RE

Based on the available studies on Repeated dose and Reproductive toxicity there is no indication for specific target organ toxicity after repeated exposure (STOT RE). Effects observed in kidney are limited to rats, and are not present in mice, dogs or rabbits. Therefore, its relevance to humans is also limited and classification in this hazard class is not appropriate. Furthermore, these effects take place only in the presence of decreased water intake due to the palatability of the substance. Studies conducted by gavage do not show such a decrease in water intake and the effects on the kidney are comparable to those of any other organ or do not show a dose-response relationship. For these reasons, effects in kidney are considered an adaptive response to water intake that is not considered toxicologically relevant. Neither is renal function affected (ie, urinalysis does not show abnormal values).

No human data addressing specific target organ toxicity after repeated exposure (STOT RE) is available.

No other studies relevant for specific target organ toxicity after repeated exposure (STOT RE) are available.

A.3.7.4.2 Comparison with the CLP criteria

Based on the available studies on Repeated dose and Reproductive toxicity there is no indication for specific target organ toxicity after repeated exposure (STOT RE) and no further studies are available addressing specific target organ toxicity of Bronopol in animals or humans are available. Thus, no NOAEL and LOAEL can be derived for STOT RE, and classification and labelling for STOT RE 1 or 2 according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.7.4.3 Conclusion on classification and labelling for STOT RE

Based on the available studies on Repeated dose and Reproductive toxicity there is no indication for specific target organ toxicity after repeated exposure (STOT RE) and no further studies are available addressing specific target organ toxicity of Bronopol in animals or humans are available. Thus, no NOAEL and LOAEL can be derived for STOT RE, and classification and labelling for STOT RE 1 or 2 according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.8 Genotoxicity / Germ cell mutagenicity

A.3.8.1 *In vitro*

Table A-32: Summary table of in vitro genotoxicity studies

Method, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains)	Results (including cytotoxicity and +/-S9 mix)	Remarks (e.g. major deviations)	Reference
Bacterial gene mutation OECD 471, GLP Rel. 1 Supportive	Bronopol (98.7%) Concentrations: 1, 2, 4, 8, 16, 32, 64 µg/plate	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102	**Negative** in the absence and presence of S9-mix. Cytotoxicity plate incorporation test: cytotoxicity at 64 µg/plate for TA 1535, TA 100 (-S9) pre-incubation test: cytotoxicity at 64 µg/plate for TA 100, TA 1537, TA 98, TA 102 (-S9), ≥32 µg/plate for TA 1535 (-S9)		[redacted] 2000 A6_06_1-1
Ames test (according to Ames *et al*. (1975) Proc. Nat. Acad. Sci. 70: 2281-2285 & 782-786, 1973 and Mutat. Res. 31: 347-364 preceding OECD guideline 471) GLP Rel. 1 Supportive	Bronopol (99.7%) Concentrations: 3.9, 7.8, 15.6, 31.2, 62.5, 125 µg/plate	*S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100	**Negative** in the absence and presence of S9-mix. Positive and negative controls were included in the test. Cytotoxicity was observed at 125 µg/plate +S9, and at 62.5 µg/plate -S9. No increase in the number of revertants		[redacted] 1974 A6.06.1_01

			was observed in the + or -S9 in any of the tester strains.		
Chromosome aberration test **OECD 473,** **GLP,** **Rel. 1** **Supportive**	Bronopol (98.7%) Concentrations: -S9: 3, 5, 7, 9, 11 µg/mL +S9: 10, 15, 20, 23, 26 µg/mL	Chinese hamster V79 cells	**Positive** in the absence and presence of S9-mix. Cytotoxicity at ≥5 µg/mL (18 h, -S9), ≥7 µg/mL (30 h, -S9), ≥20 µg/mL (18 h, +S9), and ≥23 µg/mL (30 h, +S9) In the absence of metabolic activation, after 30 h incubation there was a statistical significant increase at 7 µg/mL in the number of metaphases with aberrations (+/- gaps) and metaphases with exchanges (at 49 % cytotoxicity), higher dose levels were not evaluated for metaphases due to excessive toxicity (cytotoxicity above 60%). In the presence of metabolic activation after 18 h incubation, there was a statistical significant increase in the number of metaphases with aberrations (with and		[redacted] 2000 A6_06_2-1

			without gaps) at 23 µg/mL (at 46 % cytotoxicity) and 26 µg/mL was not further evaluated due to excessive toxicity, after 30 h incubation in the presence of metabolic activation the number of metaphases with aberrations (with and without gaps) metaphases with exchanges (at 44 % cytotoxicity) were increased statistically significant as well.		
Chromosome aberration test, similar to OECD 473, GLP **Rel. 2** **Supportive**	Bronopol (99.7%) <u>Concentrations</u> -S9: 10, 20, 30 µg/mL +S9: 20, 30, 40 µg/mL	Human lymphocytes	**Negative** in the presence and **positive** in the absence of S9-mix. Positive and negative controls were included in the test. <u>Cytotoxicity</u>: > 30 µg/mL In the absence of metabolic activation, a small but statistically significant increase in the incidence of cells bearing chromosomal aberrations (with and without gaps) was evident at the maximum tested concentration of 30		[redacted] 1974 A6.06.2_01

			µg/mL Bronopol, which was confirmed in a repeat test and observed at approx. 15 to 41 % cytotoxicity (Mitotic index 59% and 85% of control in the first and repeat experiment, respectively). No such increase was seen in the presence of metabolic activation.		
Chromosome aberration test, similar to OECD 473 **GLP** **Rel. 2** **Supportive**	Formaldehyde (38%) Concentrations: 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 µg/mL In order to simulate conditions similar to those used for Bronopol testing (see A6.06.2_01), which resulted in a positive finding, the present test was conducted in the absence of metabolic activation, i.e. without S9 mix.	Human lymphocytes	**Negative** in the presence and **positive** in the absence of S9-mix. Cytotoxicity was not considered since tested formaldehyde concentrations were intended to cover the concentration range of formaldehyde resulting from hydrolysis of Bronopol at a concentration of 30 µg/mL. A statistically significant increase in chromosomal aberrations (with and without gaps) was seen at 6 and 8 µg/mL. 8% and 21% cells with aberrations (incl. gaps) were reported for 6 and 8 µg/mL respectively,	This supports the assumption that the clastogenicity reported for Bronopol (see A6.06.2_01) rather was due to released formaldehyde than to the parent compound as such.	[redacted] 1974 A6.06.7_01_a, A6.06.7_01_b

			versus 1% in the negative control. 15% of cells with aberrations (excl. gaps) were reported for 8 µg/mL, versus 0% in the negative control. The extent and quality of the findings seen at 8 µg/mL were very similar to those reported for Bronopol 30 µg/mL (A6.06.2_01).		
Gene mutation in mammalian cells **OECD 476,** **GLP** **Rel. 2** **Supportive**	Bronopol (98.7%) <u>Concentrations</u>: Main test: -S9: 1, 2, 4, 8, 10, 12 µg/mL +S9: 3, 6, 12, 18, 21, 24, 27 µg/mL Confirmatory test: -S9: 3, 6, 9, 12, 15, 18, 21 µg/mL +S9: 6, 9, 12, 18, 21 µg/mL	Chinese hamster V79 cells	**Equivocal** in the presence and **positive** in the absence of S9-mix. In presence of S9 a trend towards increased values was also seen mainly in the 1st experiment, which was considered to represent an equivocal response. In the absence of metabolic activation, a statistically significant increase in the number of mutant frequencies was shown at 6, 9, 12 and 15 µg/mL Bronopol (only in the second of two trials). While at 6 and 9 µg/mL there was no relevant cytotoxicity detected,		[redacted] 2001 A6_06_3-1

			at 12 and 15 µg/mL survival was reduced to 55-35 % (45-65% cytotoxicity). In the presence of metabolic activation, a statistically significant increase in the number of mutant frequencies was observed at 6, 12 and 18 µg/mL Bronopol (only in the first of two trials). While at 6 and 12 µg/mL, there was no marked cytotoxicity (survival at 100 to 92 %), survival at 18 µg/mL was significantly reduced (replicates: 30 and 5% survival, even exceeding the anticipated cytotox limit of 90 % at one replicate), however this was concluded to be an equivocal result. Cytotoxicity at ≥15 µg/mL (-S9), and ≥18 µg/mL (+S9)		
Mammalian cell gene mutation assay (according to Mc Millan S and Fox M (1979) Mut. Res. 60: 91-107, with some modifications) **GLP** **Rel. 2**	Bronopol (99.7%) Concentrations: Main test: -S9: 0.5, 1, 2, 4, 8, 16 µg/mL +S9: 1, 2, 4, 8 µg/mL Confirmatory test:	Chinese Hamster V79 cells	**Negative** in the absence and presence of S9-mix. Positive and negative controls were included in the test. Cytotoxicity:		[redacted] 1974 A6.06.3_01

Supportive	+ S9: 4, 5, 6, 7, 8 µg/mL		-S9 mix: 16 µg/mL. +S9 mix: 8 µg/mL.		

Conclusion used in Risk Assessment – Genotoxicity in vitro	
Conclusion	Bronopol is considered inconclusive in the available *in vitro* tests.
Justification for the conclusion	No mutagenic activity of Bronopol was observed in bacterial strains of *Salmonella typhimurium* (Ames test) whereas Bronopol is considered inconclusive with regard to genotoxicity and gene mutation in mammalian cells (Chinese hamster cells V79). It was shown that Bronopol is non-clastogenic to human lymphocytes *in vitro* (chromosome aberration test). The slightly positive effects observed in a chromosomal aberration test in cultured lymphocytes in the highest concentration tested were found to be related to released formaldehyde under cell culture conditions, indicating that this weakly positive result may be linked to the testing conditions triggering the release of formaldehyde from Bronopol. In consequence, the biological relevance of the *in vitro* results for the evaluation of Bronopol are ambiguous and further assessed in related *in vivo* studies.

Genotoxicity of bronopol *in vitro* was assessed in different test systems performed according to scientifically valid methods preceeding the OECD test guidelines.

In a gene mutation study in bacteria ([redacted] 1974; A6.06.1_01), strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 of *Salmonella typhimurium* were exposed to 3.9, 7.8, 15.6, 31.2, 62.5 or 125 µg/plate, with and without metabolic activation. Cytotoxicity was observed at 125 µg/plate in the presence of metabolic activation and at 62.5 µg/plate in the absence of metabolic activation. The numbers of revertants recorded were within the range of the negative control and the positive controls increased numbers of revertants, so bronopol showed no mutagenic potential in the Ames test with and without metabolic activation.

The potential of mutagenicity of Bronopol was assayed in an Ames test ([redacted] 2000; A6_06_1-1) in absence or presence of metabolic activation using different strains of *S. typhimurium*. Marked cytotoxicity was detected at 158 µg/plate. No mutagenicity was seen in this test in absence or presence of S9 using different strains of *S. typhimurium.*

The clastogenicity potential of bronopol against mammalian cells was assessed using Chinese hamster V79 cells ([redacted] 1974; A6.06.3_01). These cells were exposed to 0.5, 1, 2, 4, 8 or 16 µg/mL in the absence of S9 mix and 1, 2, 4 or 8 µg/mL in the presence of S9 mix. The maximum concentration for mutagenicity testing allowed by the cytotoxicity of bronopol was 8 µg/mL. In the absence of S9 mix, no evidence of mutagenicity was seen at test concentrations of bronopol up to 8 µg/ml. In the presence of S9 mix, an increase in mutant frequency was seen in the first test but it was within control range. Therefore, it is concluded that bronopol was not mutagenic in Chinese hamster V79 cells.

An *in vitro* gene mutation test was assayed in Chinese hamster V79 cells ([redacted] 2000; A6_06_3-1), under guidelines. The mutagenicity test was performed twice, at 0-27 µg/mL the first experiment, and 0-21 µg/mL the second experiment. The cytotoxicity test was performed

at 0-30 µg/mL. Bronopol showed an increase in the mutant frequency in absence in metabolic activation in both experiments. However, in presence of metabolic activation, the increase of mutant frequency in the first experiment was not reproducible in the second experiment. Therefore, bronopol was mutagenic in –S9 with an equivocal response in +S9. Bronopol also had cytotoxicity in mammalian cells (≥15 µg/mL and ≥18 µg/mL, in –S9 and +S9 respectively). Some positive responses *in vitro* occurred at dose levels that also exhibited cytotoxicity.

Bronopol was tested in the chromosomal aberration assay using human lymphocytes ([redacted] 1974; A6.06.2_01). Dose levels for the assay were 10, 20 and 30 µg/mL without S9, and 20, 30, and 40 µg/mL with S9. A small but statistically significant increase in the incidence of cells showing chromosomal aberrations was evident at the maximum tested concentration of bronopol in the absence of S9 mix. No increased incidence of cells with chromosomal aberrations was seen in the presence of S9 mix. It was suggested that the observed clastogenic effect rather might have been due to formaldehyde liberated from bronopol-degradation, than to bronopol as such.

The concentration of formaldehyde resulting from degradation of bronopol in the cell culture medium used for the human lymphocytes under the conditions of the chromosome aberration test (CAT) was determined ([redacted] 1986; A6.10_01_a and b). Starting from an initial bronopol concentration of 30 µg/mL, about 10% were recovered in the medium after 2 to 24 h. Maximum concentration of 4.2 µg/mL formaldehyde in the test medium was reached after 2 h of incubation, then the concentration of formaldehyde decreased slightly over time. Decrease of formaldehyde concentration may be related to its volatility, or to its reaction with bronopol to form 2-hydroxymethyl-2-nitropropane-1,3-diol.

Consequently, the clastogenicity of formaldehyde in cultured human lymphocytes was assessed in the absence of metabolic activation, at a concentration range covering the 4.2 µg/mL formaldehyde shown to be released by bronopol under the test conditions of the *in vitro* CAT ([redacted] 1974; A6.06.7_01_a, A6.06.7_01_b). A statistically significant increase in chromosomal aberrations (with and without gaps) was seen at both highest test doses of 6 and 8 µg/mL formaldehyde, and the extent and quality of the findings seen at 8 µg/mL were very similar to those reported previously for bronopol. These findings support the assumption that the clastogenicity reported for bronopol was due to released formaldehyde rather than to the parent compound as such.

A CAT was performed in mammalian cells (Chinese hamster V79). This test ([redacted] 2000; A6_06_2-01) showed an increase of aberrant metaphases. In absence of metabolic activation, an increase of aberrant and polyploid metaphases was seen at 7 µg/mL, after 30 h of incubation. In presence of metabolic activation, the increase of aberrant metaphases occurred at 23 µg/mL after 18 h of incubation. Concerning the cytotoxicity, the survival indices were <10% than control at 15 mg/mL bronopol and higher concentrations. These results show that Bronopol is considered clastogenic in presence and absence of metabolic activation.

A.3.8.2 *In vivo*

Table A-33: Summary table of in vivo genotoxicity studies

Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study	**Test substance (including purity), Vehicle, Doses**	**Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure)**	**Main effects, Observations (specify regarding dose and sampling time)**	**Remarks (e.g. major deviations)**	**Reference**
Mouse bone marrow micronucleus test OECD 474, GLP Rel. 1 Supportive	Bronopol (98.7%) Vehicle: Physiological saline Doses: 0, 3, 6, 12 mg/kg bw	Mouse Hsd/Win: NMRI 5 males/group Treatment: two intra-peritoneal applications (24 h between applications) Sampling: 24 h after last treatment	**Negative** (no increase in micronucleated polychromatic erythrocytes)	(2x) 12 mg/kg bw considered as i.p. MTD in male mice	[redacted] 2001 A6_06_4-1
Mouse bone marrow micronucleus test OECD 474, GLP Rel. 1 Supportive	Bronopol (98.6%) Vehicle: Distilled water Doses: 0, 25 (males only), 50, 100, 200 (females only) mg/kg bw	Mouse CD-1 6 sex/sampling time Treatment: two oral applications (24 h between applications) Sampling: 24 h after last treatment	**Negative** (no increase in micronucleated polychromatic erythrocytes)	(2x) 100 and 200 mg/kg bw considered as i.p. MTD in male and female mice	[redacted] 2001 A6_06_4-2
Mouse micronucleus assay OECD 474, GLP Rel. 1 Supportive	Bronopol (99.7%) Vehicle: Purified water Doses: 80 and 160 mg/kg bw	Mouse, CD-1, male and female 12 animals/sex in the negative control, positive control, low dose groups 24 animals/sex in the high dose group	**Negative** (the number of polychromatic erythrocytes containing micronuclei was within the range of the negative control)	160 mg/kg bw was considered the MTD.	[redacted] 1986 A6.06.4_01

Method, duration of study, Guideline, GLP status, Reliability, Key / supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure)	Main effects, Observations (specify regarding dose and sampling time)	Remarks (e.g. major deviations)	Reference
		Treatment: single oral, gavage 24, 48 and 72 h following treatment, 4 animals/sex from the low dose group and 8 animals/sex from the high dose group were sacrificed, and the femoral bone marrow was extracted. Parameters considered: Ratio of polychromatic erythrocytes to normochromatic erythrocytes, number of micronuclei in 1000 polychromatic erythrocytes per animal.	Positive and negative controls were included in the test. Signs of toxicity: In the high dose group, 4 male and 4 female mice died within 48 h after dosing. In the low dose group, one female died within 72 h after dosing.		
UDS test in rat hepatocytes OECD 486, GLP Rel. 1 Supportive	Bronopol (98.5%) Doses: 0, 100, 200 mg/kg bw	Rat Fischer 344 4 (positive control), 7 (high dose), 5 (other), males/sampling time Treatment: Single oral application Sampling times: 2-4 and 14-16 h	**Negative** at 2-4 h and 14-16 h. (no increase in net nuclear grain counts)	200 mg/kg bw considered as oral MTD in male rats	[redacted] 2001 A6_06_5-1
UDS test in rat	Bronopol (99.5%)	Rat, Wistar, male	**Negative** at 2-4 h	150 mg/kg bw	[redacted] 1998

Method, duration of study, Guideline, GLP status, Reliability, Key / supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure)	Main effects, Observations (specify regarding dose and sampling time)	Remarks (e.g. major deviations)	Reference
hepatocytes **OECD 486,** **GLP** **Rel. 1** **Supportive**	Doses: 0, 60, 150 mg/kg bw	5 (controls) and 25 (mid and high dose) males/sampling time Treatment: i) Oral, gavage, single application. Sampling times: 2-4 and 12-14 h	and 12-14 h. (no increase in net nuclear grain counts)	considered as oral MTD in male rats	A6.06.5_01
Dominant lethal assay (according to Bateman AJ (1958) Heredity 12: 213-232, and Bateman AJ and Epstein SS, in Chemical Mutagens, Vol. 2, ed. Hollaender, Plenum Press, 1971) **Non-GLP** **Rel. 2** **Key**	Bronopol, purity not specified. Doses oral treatment: 20 and 100 mg/kg Dose for i.p. treatment: 10 mg/kg bwin 0.9% saline.	Mouse, OLAC, male and female 10 males/group (only males were treated) Treatment: i) Oral, gavage, daily for 6 consecutive days. ii) single i.p. injection After treatment each male was housed with 3 females for mating, which were replaced at weekly intervals for 4 weeks. The females were killed 14 days from the midpoint of the mating week, corresponding to gestation days 9 to 16. Numbers of pregnancies, and live	**Negative** Positive and negative controls were included in the test. Signs of toxicity: Four of the 10 males treated orally with 100 mg/kg bw Bronopol died. One male from the i.p. treated group died. Oral dosage: In the high dose group, the total number of implants per female was significantly reduced in weeks 2 and 3		[redacted] 1974 A6.06.6_01

Method, duration of study, Guideline, GLP status, Reliability, Key / supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure)	Main effects, Observations (specify regarding dose and sampling time)	Remarks (e.g. major deviations)	Reference
		and dead implants were recorded	accompanied by a decreased number of live implants and a reduced number of pregnancies. For both tested doses, no increase in the frequency of dead implants was observed. I.p. injection: The implantation rate was significantly reduced in week 4. Fertility was reduced as indicated by a decreased pregnancy rate. A decrease in the frequency of dead implants was seen after 4 weeks. Overall result: No increase in post-implantation loss has been observed in any of the groups treated with Bronopol.		

No human data on genotoxicity is available.

Genotoxicity of bronopol *in vivo* was assessed in three different test systems performed according to scientifically valid methods following the OECD test guidelines, except for the dominant lethal assay.

CD1 mice were treated orally with a single dose of 0, 80, or 160 mg/kg bw/day, with a positive control ([redacted] 1986; A6.06.4_01). All groups included 12 animals/sex/group except the highest dose group, including 24 animals/sex. In the 160 mg/kg bw group, 4 males and 4 females died within 48 h. In the 80 mg/kg bw group, 1 female died within 72 h. The incidence of micronuclei in polychromatic erythrocytes of bronopol-treated mice was within the range of negative control. In the positive control (cyclophosphamide), significant increases in the incidence of micronuclei in polychromatic erythrocytes were reported after 24 and 48 h. After 72 h, the incidence of micronuclei in polychromatic erythrocytes was increased in the males but without being statistically significant. These data indicate that bronopol at doses up to 160 mg/kg bw, which is the MTD for mice, was not clastogenic in the *in vivo* mouse micronucleus test.

Two micronucleous test (with intraperitoneal and oral application) were performed in mice. In the intraperitoneal micronucleous test ([redacted] 2001; A6_06_4-1) 2 applications of 0, 3, 6 or 12 mg/kg bw were given to 5 male mice/dose. Clinical signs like apathy or difficulty in breathing appeared at all dose levels. No mortality was observed. The number of micronucleated polychromatic erythrocytes was not affected, so bronopol was not considered genotoxic. No indications for clastogenicity *in vivo* were seen.

In an oral mouse bone marrow micronucleous test ([redacted] 2001; A6_06_4-2) 6 mice/sex/concentration were administered 0, 25, 50 or 100 mg/kg bw/day to the males and 0, 50, 100 or 200 mg/kg bw to the females. 3 females died and clinical signs like decreased activity and laboured respiration appeared. This test did not show effect in the number of micronucleated polychromatic erythrocytes. However, a slight change in the ratio between polychromatic erythrocytes and normochromatic erythrocytes was observed in females at the highest dose. These results conclude that bronopol was not genotoxic in this study.

In an *in vivo* genotoxicity study ([redacted] 1998; A6.06.5_01), groups of Wistar rats were administered doses of bronopol at concentrations of 0, 60 or 150 mg/kg bw, with two positive controls (2-acetamidofluorene or dimethylnitrosamine). For the animals of the 150 mg/kg bw groups, abnormal gait and breathing were reported. 1 animal in the 60 mg/kg bw group showed similar symptoms. No mortality was seen. The mean net grain count values for the bronopol treated groups ranged between –1.6 and –2.0, below the threshold value indicative of a positive response (0). The oral treatment of male rats with bronopol at doses up to 150 mg/kg bw, which was the MTD, did not induced increased UDS in the hepatocytes of the liver. The test substance showed no genotoxic potential.

The *in vivo* unscheduled DNA synthesis (UDS) test in rat (hepatocytes) ([redacted] 2001; A6_06_5-1) for DNA damage, including gene mutations did not indicate signs of genotoxicity. The nuclear grain count and the percentage of cells in repair were unaffected. Therefore Bronopol was non-genotoxic *in vivo*.

A dominant lethal assay was performed in mice ([redacted] 1974; A6.06.6_01). OLAC mice were given orally 0, 20, 100 mg/kg bw or 10 mg/kg bw by an intraperitoneal injection with a positive control (tris(2-methyl-1-aziridinyl)-phosphine oxide). Mortalities in males were seen at the highest orally applied dose of bronopol (100 mg/kg bw). For the highest orally applied dose of bronopol, and for the single i.p. injection, a decrease in the pregnancy rate was observed. Implantation rates were significantly reduced in week 2 and 3 for the group treated orally with 100 mg/kg bw. For the group having received i.p. injection a similar reduction was observed and an increase in the frequency of dead implants in week 4. These effects were a consequence of the toxicity of the tested concentrations on the male mice. Therefore, they rather were seen as non-genetic anti-fertility effects and were not indicative of a cytogenic effect of bronopol on the meiotic and post-meiotic sperm stages.

A.3.8.2.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Genotoxicity of Bronopol *in vitro* was assessed in three different test systems performed according to scientifically valid methods following OECD TGs. No mutagenicity of Bronopol was observed in five strains of *Salmonella typhimurium* with and without metabolic activation ([redacted] 2000 (A6_06_1-1) and [redacted] 1974 (A6.06.1_01)).

Results of mammalian cell gene mutation tests in Chinese hamster V79 cells according to OECD TG 476 ([redacted] 2000; A6_06_3-1) exhibited equivocal results in the presence and positive results in the absence of metabolic activation. In another study ([redacted] 1974; A6.06.3_01) it was shown that no forward mutation in the HPRT locus in the presence and absence of metabolic activation was observed when tested up to a comparable concentration of Bronopol (16 and 15 µg/mL in the absence of metabolic activation).

A slight increase in the number of cultured human lymphocytes with chromosomal aberrations (with and without gaps) has been observed after exposure to Bronopol at the maximum tested concentration of 30 µg/mL Bronopol in the absence of metabolic activation ([redacted] 1974; A6.06.2_01).

Subsequently, the concentration of formaldehyde resulting from degradation of Bronopol in the cell culture medium used for the human lymphocytes under the conditions of the chromosome aberration test (CAT) was determined ([redacted] 1986; A6.10_01_a and b). Starting from an initial Bronopol concentration of 30 µg/mL, about 10% were recovered in the medium after 2 to 24 h. Maximum concentration of 4.2 µg/mL formaldehyde in the test medium was reached after 2 h of incubation, then the concentration of formaldehyde decreased slightly over time. Decrease of formaldehyde concentration may be related to its volatility, or to its reaction with Bronopol to form 2-hydroxymethyl-2-nitropropane-1,3-diol. Consequently, the clastogenicity of formaldehyde in cultured human lymphocytes was assessed in the absence of metabolic activation, at a concentration range covering the 4.2 µg/mL formaldehyde shown to be released by Bronopol under the test conditions of the *in vitro* CAT (in [redacted] 1974; A6.06.7_01_a and b). A statistically significant increase in chromosomal aberrations (with and without gaps) was observed at both highest test doses of 6 and 8 µg/mL formaldehyde, and the extent and quality of the findings seen at 8 µg/mL were very similar to those reported previously for Bronopol ([redacted] 1974; A6.06.7_01_a and b). These findings support the assumption that the clastogenicity reported for Bronopol was due to released formaldehyde rather than to the parent compound as such. In general, due to the release of formaldehyde under culture conditions *in vitro*, the *in vitro* results for Bronopol are questionable and are thus further assessed *in vivo*.

Genotoxicity of Bronopol *in vivo* was assessed in three different test systems. Micronucleus test in mice and the UDS test in rats followed OECD test guidelines while the dominant lethal test was performed according to a scientifically valid methods similarly to the OECD test guideline. In all three test systems the highest dose of Bronopol applied orally caused overt signs of toxicity including mortality. Effects seen in the dominant lethal test were considered to be related to the general toxicity of Bronopol since the number of dead implants/rat was not increased. Results from all three test systems give no indication that Bronopol causes genotoxic effects *in vivo*, both in somatic and germ cells.

A.3.8.2.2 Comparison with the CLP criteria

Overall, the biological relevance of the *in vitro* results for the evaluation of Bronopol are highly questionable and further assessed in related *in vivo* studies. Results from three *in vivo* test systems (Micronucleus assay, USD test in rat hepatocytes and Germ cells Dominant lethal

assay) give no indication that Bronopol causes clastogenicity/DNA damage *in vivo.* Effects observed in the dominant lethal test were considered to be related to the general toxicity of Bronopol (at high dose levels) since the number of dead implants per rat was not increased. Thus, Bronopol causes no germ cell mutagenicity.

It can be concluded that based on available data Bronopol has no genotoxic effects *in vivo*, both in somatic and germ cells. Therefore, classification and labelling for mutagenicity should not be required.

A.3.8.2.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the available *in vivo* studies Bronopol is not genotoxic (both in somatic and germ cells) and classification and labelling for Genotoxicity/Germ cell mutagenicity according to CLP criteria should not be required under Regulation (EC) No 1272/2008.

A.3.8.2.4 Overall conclusion on genotoxicity related to risk assessment

Not applicable for the CLH report.

A.3.9 Carcinogenicity

Table A-34: Summary table of carcinogenicity studies in animals

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	**Species, Strain, Sex, No/Group**	**Test substance (including purity), Vehicle, Dose levels, Duration of exposure**	**NOAEL, LOAEL**	**Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels)**	**Remarks (e.g. major deviations)**	**Reference**
Non-guideline 104 weeks (oral via drinking water) Non-GLP Rel. 2 Key	Rat, Sprague-Dawley, male and female, Main test: 45/sex/group Satellite group for blood sampling/clinical pathology: 15/sex/group	Bronopol (purity not stated) Dose levels nominal: 10, 40, 160 mg/kg bw/day Re-evaluated doses: 7, 32, 142 mg/kg bw/day Treatment: oral, continuously via	NOAEL = 10 mg/kg bw/day (due to reduced food consumption and body weight gain) Carcinogenicity: NOAEL > 160 mg/kg bw/day (since no indication for	Systemic toxicity: Treatment- and dose-related effects seen at 40 and 160 mg/kg bw/day (reduction in food consumption and body weight gain; additional findings in the high dose group: increased mortality, reduction in grooming activity) Effects due to		A6.07_01_a [redacted] (reported in [redacted] 1976 and US EPA RED 1995), [redacted] 1993 A6.07_01_b, [redacted] 1985 A6.07_01_c, [redacted] 1998 A6.07_01_d, [redacted] 1986 A6.07_01_e,

		drinking water Duration of exposure: 104 weeks	carcinogenicity was observed up to the highest tested dose level)	palatability: In all treatment groups reduced water uptake was observed, resulting in decreased urine production and further associated with exacerbated incidence of glomerulonephrosis, which is regarded as a spontaneous occurring lesion in the used rat strain. Neoplastic finding: Squamous cell papillomata in stomach of high dose rats that died during the experiment. Squamous cell papillomata associated with epithelial hyperplasia and ulceration. The findings were rather related to the irritant potential of Bronopol than indicative of a tumorigenic potential for this substance. With regard to neoplastic changes, incidental/spontaneous findings were reported, which occurred in both treated and untreated animals, and/or showed no dose-response relationship.		[redacted] 1985 A6.07_01_f

				Overall result: Bronopol tested orally in rats over a period of 104 weeks was not carcinogenic.		
Non-guideline **80 weeks (dermal)** **Non-GLP** **Rel. 2** **Supportive**	Mouse CFLP mice Males and females 52/sex/dose	Bronopol (98 - 102%) Vehicle: 90% acetone in water Dose levels: 0, 0.2%, 0.5% corresponding to 0, 20, 50 mg/kg bw/day (assuming a body weight of 30 g/mouse) Treatment: one application/day, 3 days/week (i.e. on Monday, Wednesday and Friday) Duration of exposure: 80 weeks	NOAEL = 0.2% (20 mg/kg bw/day) (due to decreased mean body weight gain) Carcinogenicity: NOAEL > 0.5% (50 mg/kg bw/day) (since no indication for carcinogenicity was observed up to the highest tested dose level)	Systemic toxicity: A slight but statistically significant decrease in mean body weight gain was reported for the males treated with 0.5% Bronopol for the period ranging from week 26 to week 52. Some mice of the group treated with 0.5% Bronopol showed slight loss in hair around the treated skin area of mice during the first 3 weeks of treatment. No further treatment-related effects were seen. Mortality for the treated females (both test groups) was within control range and therefore inconspicuous During gross pathology and histopathology, all reported findings were incidental and without relationship to the treatment with Bronopol. Neoplastic findings: An increased incidence of skin papilloma was reported for the		A6.07_02_a (reported in [redacted] 1975 and US EPA RED 1995), [redacted] 1986 A6.07_02_b, [redacted] 1992 A6.07_02_c, [redacted] 1998 A6.07_02_d, [redacted] 1973 A6.07_02_e

				highest tested concentration of Bronopol (0.5%); however, these tumours rather resulted from the irritant potential of Bronopol than from a carcinogenic potential of Bronopol. Therefore, a carcinogenic potential could not be evidenced for Bronopol under the test conditions used. Overall result: A carcinogenic potential could not be evidenced for Bronopol under the test conditions used.		

No human data on carcinogenicity is available.

No other studies relevant for carcinogenicity are available.

A.3.9.1 Short summary and overall relevance of the provided information on carcinogenicity

For carcinogenicity a publication is available ([redacted] 1978: [redacted] 1976 (A6.07_01_a) and [redacted] 1975 (A6.07_02_a)) where the two carcinogenicity studies are described briefly and both studies are also described in more detail in the US EPA RED document.

Carcinogenicity of Bronopol was assessed in a combined chronic toxicity/carcinogenicity study after oral to rats.

Male and female Sprague-Dawley rats were exposed orally to Bronopol in acidified drinking water at concentrations designed to provide 0, 10, 40, and 160 mg/kg bw/day ([redacted] 1976; A6.07_01_a). Re-evaluation of the provided doses considering the stability of Bronopol suggested that the actual doses were 7, 32, and 142 mg/kg bw rather than the intended 0, 10, 40, and 160 mg/kg bw/day. At the end of the exposure period all animals were examined for systemic toxicity and neoplastic changes. Non-neoplastic effects related to treatment with Bronopol have been summarized in chapter A 3.7.3 (Long-term repeated dose toxicity) of this dossier. The most frequently observed

tumors in this study were pituritary adenoma in male and female rats and mammary fibroadenoma in the females. For both tumors the incidences lacked a dose-response relationship. Neoplastic changes identified by histopathological examination of the stomach of high dose rats that died during the exposure period were squamous cell papilloma associated with epithelial hyperplasia and ulceration. A more recent re-evaluation of the histopathological findings confirmed these findings and papillomas were also reported for the forestomach of the rats treated with Bronopol. However, the fact that these findings mainly were seen in the high dose group and were associated with ulceration is taken as indication they were a consequence of the irritant potential of Bronopol rather than reflecting a carcinogenic potential of the test substance. Thus, no carcinogenic potential could be evidenced for Bronopol when applied orally under the test conditions chosen.

Moreover, carcinogenicity of Bronopol was assessed in mice in a combined chronic toxicity/carcinogenicity study after dermal exposure. Bronopol is applied dermally in concentrations 0.2% and 0.5% to male and female CFLP mice ([redacted] 1975; A6.07_02_a), primarily focussing on the potential carcinogenicity resulting from treatment with Bronopol. Two skin tumours (skin papilloma) were seen in the control group (2/104), with one of them having regressed subsequently; five animals bearing skin papilloma were seen in the 0.5% Bronopol test group (5/104), with one of them having regressed subsequently. However, the statistical assessment of the difference between the 0.5% Bronopol test group and the control revealed no statistical significance. With regard to the number of mice bearing tumours, there was no conspicuous difference between the Bronopol treated and the control groups. As the study was conducted in the seventies, a re-evaluation of the histopathological findings was undertaken in the early nineties, which confirmed the incidence of skin papillomas on the treated skin site of one male and three females in the high dose group; after further examination of these papillomas these tumours were considered to result from the local irritancy of Bronopol. Thus, no carcinogenicity of dermally applied Bronopol in mice was observed under the test conditions chosen.

An increased incidence of lymphoma was reported for the females treated with bronopol; however, the difference from control was not statistically significant and the incidence seen in the treated animals was within the normal range. Two skin tumours were seen in the control group, with one of them having regressed subsequently and five skin tumours were seen in the 50 mg/kg bw/day test group, with one of them having regressed subsequently. However, differences between the 50 mg/kg bw/day test group and the control were without statistical significance.

No malignant tumours were found in the treated skin areas of the test animals, and no further tumours were considered to be treatment-related. The examination of the four skin papillomas seen in the 50 mg/kg bw/day test group led to the conclusion that these tumours rather were related to the irritant potential than to a carcinogenic potential of bronopol. Therefore, a carcinogenic potential could not be evidenced for bronopol under the test conditions used.

Conclusively, no treatment-related significant increase in neoplastic findings was reported after chronic oral and dermal exposure of rats and mice to Bronopol. Thus, Bronopol is considered not carcinogenic.

A.3.9.2 Comparison with the CLP criteria

Based on the available data and in the absence of genotoxicity *in vivo*, Bronopol is considered to be non-carcinogenic. Therefore, classification and labelling for carcinogenicity should not be required.

A.3.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data and in the absence of genotoxicity *in vivo*, Bronopol is considered to be non-carcinogenic. Therefore, classification and labelling for carcinogenicity should not be required.

A.3.9.4 Overall conclusion on carcinogenicity related to risk assessment

Not applicable for the CLH report.

A.3.10 Reproductive toxicity

A.3.10.1 Sexual function and fertility

Table A-35: Summary table of animal studies on adverse effects on sexual function and fertility

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
OECD 416, Oral (via drinking water) GLP Rel. 1 Key	Rat Crl:CD(SD) 27 sex/ dose/ generation	Bronopol (98.85%) Dose levels: 0.01, 0.05, 0.15% equivalent to target doses of 10, 50, and 150 mg/kg/day of test material Exposure period: P1/P2 adults: 10	NOAEL (F0), systemic toxicity = 10 mg/kg bw/day NOAEL (F1), systemic toxicity = 50 mg/kg bw/day NOAEL (F0, F1), reproduction and fertility = 50 mg/kg bw/day	Critical effects: P1/P2 systemic: ↓ bw gain (gestation), ↓ water consumption (palatability), ↑ rel. kidney and thyroid weight, minor microscopic changes in kidneys, thyroid, stomach and liver P1/P2		[redacted] 2008 A6_08_2-1

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
		weeks pre-mating, up to 2 weeks mating, gestation, lactation		reproductive: increased postimplantation loss, decreased gestation survival only at the high dose F1/F2 pups: No effects observed		
Two-generation study according to the SOP of IRDC, Oral (drinking water) **GLP** **Rel. 1** **Supportive**	Rat, CD, male and female, 13 males and 26 females/ group	Bronopol (99.9%) Doses: 0, 25, 70, 200 mg/kg bw/day (water, pH 4.0) (Test doses derived from a range-finding study) Pre-mating: 80 days (males and females) Exposure period: from 80 days prior mating of the parental	NOAEL (F0, F1), systemic toxicity = 25 mg/kg bw/day NOAEL (F0, F1), reproduction and fertility = 70 mg/kg bw/day NOAEL (F1, F2), development = 25 mg/kg bw/day	Test substance intake for F0 and F1 males and females: 22.5, 55.2 and 147 mg/kg bw/day respectively. The lower achieved dosages of Bronopol were due to the reduced water consumption, which was observed in all treated groups. Systemic toxicity (parental F0, F1, F2): No treatment-		[redacted] 1987 A6.08.2_01_a Range-finding study: [redacted] 1986 A6.08.2_03

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
		generation (F0) until 33 to 47 days following weaning of the F2.		related mortality (cases of death were incidental) Treatment-related effects seen at all tested doses, however particularly pronounced at the highest dose tested (decrease in body weight gain, food consumption, water consumption). <u>Pathological findings</u>: Increased incidence of progressive nephropathy for some high-dose parental animals of both sexes (F0 & F1); the finding was seen as treatment-related but was not a direct effect of the test		

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
				substance as such. In high-dose F1 parents, changes in liver and body weight were treatment-related effects whereas changes in heart weight were reported as possibly treatment-related. A treatment-related decrease in mean absolute liver weight was reported for the F2b males of the high-dose group; the F2b females of the same group showed significant decreases in absolute kidney and liver weights. <u>Reproduction parameters</u>: Effects reported at the highest test		

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
				dose of 200 mg/kg bw/day such as the reduction of fertility index for the high dose parental F0 females, resulted rather from the high systemic toxicity observed at this dose level than reproductive toxicity. A minimal decrease in body weight of the F1b pups at weaning reported for the mid-dose group.		
One-generation study according to FDA Guideline, Oral (gavage) Non-GLP Rel. 2 Supportive	Rat, CD-1, male and female, 11males/group 22 females/group	Bronopol (98-102%) Doses: 0, 20, 40 mg/kg bw/day Pre-mating: males, 63 days, females, 14 days	NOAEL (F0, F1), systemic toxicity = 40 mg/kg bw/day NOAEL, reproduction and fertility = 40 mg/kg bw/day NOAEL, development =	Parental animals: 5 cases of death, however not treatment-related. No treatment-related symptoms of toxicity For the males of the 40 mg/kg bw group and starting		[redacted] 1973 A6.08.2_02

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels, Duration of exposure	NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility)	Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
		Exposure period: 19 weeks	40 mg/kg bw/day	from week 2 of treatment, weight gain slightly but clearly below control values. Reproduction parameters: All considered parameters within control range, no treatment-related effects; no treatment-related abnormalities.		

No human data addressing sexual function and fertility is available.

No other studies relevant for sexual function and fertility are available.

A.3.10.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

A two-generation reproduction toxicity study was conducted according to OECD TG 416 in Crl:CD(SD) rats at dose levels of 0, 0.01, 0.05, and 0.15% Bronopol in pH 4 acidified drinking water (equivalent to 10, 50 and 150 mg/kg bw/day) ([redacted] 2008; A6_08_2-1). Reproductive effects occurred only at the 0.15% dose level and were limited to two cases of difficult delivery (dystocia) in the P1 dams, and increased postimplantation loss (18.8 *vs*. 6.8% in controls) and associated decreases in gestation survival and stillborn pups, along with 2 total litter losses in the P2 generation. These reproductive effects were attributable to 6 high-dose dams, several of which exhibited clear signs of maternal toxicity in late gestation. There were no effects on any parameter of reproductive performance or offspring growth and survival at 0.01 or 0.05% Bronopol. Thus, the NOAEL for systemic toxicity (F0) in rats is 10 mg/kg bw/day and (F1, F2) 50 mg/kg bw/day, the NOAEL for reproduction (F0, F1) is 50 mg/kg bw/day.

Moreover, the impact of Bronopol on the fertility was assessed in rats in a former two-generation study performed according to the principles of GLP ([redacted] 1987; A6.08.2_01_a) and in a one-generation study following the FDA guideline available at that time ([redacted] 1973; A6.08.2_02).

In the two-generation study ([redacted] 1987; A6.08.2_01_a), Charles River CD rats were administered Bronopol in drinking water (pH 4.0) during the premating (80-87 days), mating, gestation and lactation periods. The two-generation study involved the parental F0 group and litters F1a and F1b, and the parent group F1 and litters F2a and F2b. F1b was selected as parent generation for F2. Bronopol was tested at doses of 0, 25, 70 and 200 mg/kg bw/day; the doses were selected on the basis of the results of a range-finding study (A6.08.2_03). Owing to water consumption, the mean achieved doses of Bronopol were calculated to be the follows: 0, 22.5, 55.2 and 147 mg/kg bw/day. With regard to systemic toxicity, effects were observed mostly in the mid-dose (70 mg/kg bw/day) and high-dose (200 mg/kg bw/day) groups, in both generations. Main effects reported for the mid dose included increased kidney weight (F0 females), decreased liver weight (F1 males and females), and increased incidence of nephropathy (F0 males: 4/10 *vs*. 2/10 in controls; F0 females: 3/10 *vs*. 0/10 in controls). Toxic effects reported for the high dose included decreased body weight (F0 and/or F1 females during the premating, gestation and/or lactation periods; F1 males), decreased food consumption (F0 males and females, F1 females), increased in organ weights (adrenals in F0 females, kidneys in F0 males and females, thyroid/parathyroid in F1 males) and decreased liver weight (F1 males). An increased incidence of nephropathy was reported for the F0 males and females (males: 6/10 *vs*. 2/10 in controls; females: 9/10 *vs*. 0/10 in controls). The low-dose animals were inconspicuous. With regard to reproductive toxicity, a slight decrease in the female fertility index during the F1 mating (75 *vs*. 87.5% in the controls) was reported for the high-dose group. Thus, the NOAEL for systemic toxicity (F0, F1) in rats is 25 mg/kg bw/day, the NOAEL for reproduction (F0, F1) is 70 mg/kg bw/day and the NOAEL for development (F1, F2) is 25 mg/kg bw/day.

In the one-generation study ([redacted] 1973; A6.08.2_02), Bronopol was given orally by gavage to Charles-River CD rats in doses of 0, 20 and 40 mg/kg bw/day. The treatment period of 19 weeks included a pre-mating exposure period of 63 and 14 days for males and females, respectively. Except for a slight decrease in body weight gain reported for the male of the 40 mg/kg bw/day group, no treatment-related effects were reported for the parental animals. All considered reproduction parameters (e.g. gestation duration, pregnancy, number of corpora lutea, pre- and postimplantation losses) were within control range and showed no treatment-related effects; examination of the offspring revealed no treatment-related abnormalities. The parental NOAEL for reproduction was 40 mg/kg bw/day for both sexes; the NOAEL for offspring also was 40 mg/kg bw/day, which was the highest dose tested.

A.3.10.1.2 Comparison with the CLP criteria

Reliable reproductive toxicity studies on Bronopol are available. In the most recent 2-generation reproductive toxicity study, reproductive effects occurred only at the highest dose level and were attributed to dams most of which exhibited clear signs of maternal toxicity during gestation. This is clearly supported by a further two-generation study, where reproductive effects were also limited to the high dose group showing clear signs of systemic/maternal toxicity. These two studies as well as the one-generation study available consistently identified no relevant adverse effects on reproductive parameters were at lower dose levels in the absence of general toxicity.

Concludingly, these data clearly indicate that Bronopol is not a selective reproductive toxicant. Therefore, classification and labelling for sexual function and fertility should not be required.

A.3.10.1.3 Overall conclusion on sexual function and fertility related to risk assessment

Not applicable for the CLH report.

A.3.10.2 Developmental toxicity, Teratogenicity

Table A-36: Summary table of animal studies on adverse effects on development

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
OECD 414, Oral (gavage), GLP Rel. 1 Key	Rabbit NZW 26 dams/ group	Bronopol (99.9%) Vehicle: acidified deionized water, *ca.* pH 4 Dose levels: 0, 3, 10, 30 mg/kg bw/day Exposure period: Day 7-27 of gestation	NOAEL maternal toxicity = 10 mg/kg bw/day NOAEL Teratogenicity Embryotoxicity = 10 mg/kg bw/day	Dams: clinical signs, ↓ bw gain and food consumption	Fetuses: slightly ↑ malformations (related to maternal toxicity)		[redacted] 2006 A6_08_1-1
EPA OPP 83-3, similar to OECD 414, Oral (gavage), GLP	Rabbit, New Zealand White, female, 18 to 20/group	Bronopol (99.8%) Doses: 0, 5, 20, 40, 80	NOAEL maternal toxicity = 40 mg/kg bw/day	Maternal Mortality: No treatment-related mortalities Clinical symptoms of toxicity:	Teratogenicity/ Embryotoxicity: Overt signs of teratogenicity were observed		[redacted] 1991 A6.08.1_01 (reported in US EPA RED 1995)

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
Rel. 1 Supportive		mg/kg bw/day (based on a range-finding study) <u>Exposure period</u>: Day 7 to 19 of gestation Sacrifice on day 28 of gestation	NOAEL developmental toxicity = 40 mg/kg bw/day Remark: Teratogenicity was observed at a test dose, which was toxic to dams. (*i.e.* 80 mg/kg bw/day)	In the high-dose group reduced size/quantity of fecal pellets, decreased food consumption, decrease in maternal body weight gain was observed. <u>Necropsy</u>: Inconspicuous	in the high-dose group only. Significant decrease in mean fetal weight for both sexes indicative of embryonic growth retardation, probably related to the decreased maternal food consumption and body weight gain. Gravid uterine weights were inconspicuous. Increase in the incidence of fetuses showing major external/visceral and skeletal abnormalities (6.9% *vs*. 0% in control) and		Range finding study: [redacted] 1991 A6.08.1_04

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
					minor skeletal abnormalities (29.5% *vs.* 10.2% in the control group). Increased incidence of fetuses with unossified forelimb (8%) and hindlimb (16%) epiphyses.		
EPA OPP 83-3, similar to OECD 414, Oral (gavage), GLP Rel. 2 Supportive	Rat, Sprague-Dawley, mated females, 24/group	Bronopol (≥99.5%) Vehicle: acidified purified water, pH 4 Doses: 0, 10, 28, 80 mg/kg bw/day (based on a range-finding study)	NOAEL maternal toxicity = 80 mg/kg bw/day NOAEL developmental toxicity = 80 mg/kg bw/day	Maternal mortality: None Clinical symptoms of toxicity: At 80 mg/kg bw/day, a significant but transient decrease in body weight gain was reported from day 6 to 7 of pregnancy; thereafter body weight gain in this group turned back to control level.	Embryonic/Fetal development: No treatment related effects on embryonic and fetal development could be evidenced. Some, advanced ossification of the sacral neural arches in the 80 mg/kg bw/day group, and		[redacted] 1995 A6.08.1_02 (reported in US EPA RED 1995)

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
		Exposure period: Day 6 to 15 of gestation Sacrifice on day 20 of gestation		Necropsy: Inconspicuous	advanced ossification of the forelimb phalanges in both the 28 and the 80 mg/kg bw/day groups were reported, but their incidences were within the range of the negative control group.		
Non-guideline dose-range finding study, Oral (gavage), GLP Rel. 2 Supportive	Rat, Sprague-Dawley, mated females, 5/group	Bronopol (≥99.5%) Vehicle: acidified purified water, pH 4 Doses: 0, 3, 10, 30, 100 mg/kg bw/day (phase I), 60, 80, 100 mg/kg bw/day	NOAEL maternal toxicity = 10 mg/kg bw/day NOAEL developmental toxicity = 100 mg/kg bw/day	Maternal mortality: 3 dams at 100 mg/kg bw/day were sacrificed *in extremis* Clinical symptoms of toxicity: Signs indicative of maternal toxicity were observed from 30 mg/kg bw/day, up to the highest test dose of 100 mg/kg bw/day. These signs mainly	No signs of developmental toxicity could be evidenced; in fact, all considered parameters were inconspicuous.		[redacted] 1993 A6.08.1_05

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
		(phase II) <u>Exposure period</u>: Day 6 to 15 of gestation Sacrifice on day 20 of gestation		consisted of a reduction in body weight gain, reduced food consumption, a poor state of health (100 mg/kg bw/day) and impaired respiration (100 mg/kg bw/day). No signs of toxicity were seen at the lowest tested doses of 3 and 10 mg/kg bw/day respectively. Necropsy of females sacrificed *in extremis*: Red lung lobes, an area of haemorrhaging of the stomach glandular mucosa and gas in the caecum, extensive ulceration within the glandular stomach mucosa and colon contents were			

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
				dehydrated Necropsy of dams at end of study: Inconspicuous			
Non-guideline, Oral (gavage), Non-GLP Rel. 2	Rat, CD, mated females, 20/group	Bronopol (98-102%) Vehicle: acidified purified water pH 4 Doses: 0, 20, 40 mg/kg bw/day (based on a preliminary test) Exposure Period: Day 15 of gestation, throughout lactation period, up to day 21 postpartum	NOAEL maternal toxicity = 40 mg/kg bw/day NOAEL developmental toxicity = 40 mg/kg bw/day NOAEL postnatal development = 40 mg/kg bw/day	The study focussed on the effect of Bronopol on the peri- and post-natal development of rat pups obtained from treated females. Maternal mortality: No treatment-related mortality was seen (2 cases of mortality were reported, however not related to the treatment with the test substance). Clinical symptoms of toxicity: None. Body weight, pregnancy rate and pregnancy duration were inconspicuous.	Litter and pup data: Litter loss: 1 dam of the control group, 2 dams of the low dose group and 1 dam of the high dose group had total litter loss. Pup mortality was slightly increased in the treatment groups from day 4; (statistically significant differences from control on day 12 and 21 for the 20 mg/kg bw/day group, and on day 21		[redacted] 1973 A6.08.1_03

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
					for the 40 mg/kg bw/day group); however, without toxicological relevance as the pup mortality in the control group was unusually low when compared to historical control data of the laboratory. Litter and mean pup weights were slightly below control values from day 12 post-partum and below the historical control data of the laboratory on day 21 in treated groups; however, the differences were		

Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study	Species, Strain, Sex, No/Group	Test substance (including purity), Vehicle, Dose levels	NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity)	Results, maternal/parental (e.g. corrected body weight gain, for all dose levels)	Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels)	Remarks (e.g. major deviations)	Reference
					of no statistical significance. Abnormalities in pups: None		

No human data addressing developmental toxicity is available.

No other studies relevant for developmental toxicity are available.

A.3.10.2.1 Short summary and overall relevance of the provided information on adverse effects on development

Developmental toxicity of Bronopol was assessed in the rat and rabbits.

The test protocols followed guideline 83-3 of the Office of Pesticide Programs (OPP), which is similar to OECD TG 414 ([redacted] 1991 (A6.08.1_01) and [redacted] 1995 (A6.08.1_02)). Bronopol was administered orally by gavage in acidified water (pH 4) to mated Sprague-Dawley rats at dose levels of 0, 10, 28 and 80 mg/kg bw/day from day 6 to 15 of gestation ([redacted] 1995; A6.08.1_02). The dose range was selected on the basis of a range-finding study, which revealed that doses >100 mg/kg bw/day administered by gavage, caused severe gastrointestinal irritation that led to death ([redacted] 1993; A6.08.1_05). A transient effect on body weight gain was reported as only sign of maternal toxicity seen at the highest dose tested. The NOAEL for maternal toxicity in the rat was 80 mg/kg bw/day. Since no developmental toxicity was seen in any of the applied doses, up to the maximum tolerable dose level, the NOAEL for developmental toxicity in the rat is 80 mg/kg bw/day.

Effects of Bronopol on the peri- and postnatal development of rat pups were assessed by oral administration of the test substance to mated

CD rats from day 15 of gestation to day 21 post-partum in a non-guideline study ([redacted] 1973; A6.08.1_03). Bronopol was administered by gavage in distilled water at doses of 0, 20 and 40 mg/kg bw/day. Neither the dams nor the pups showed any signs of toxicity related to the treatment. Thus, the NOAEL for both maternal toxicity and the peri-and postnatal development in the rat is 40 mg/kg bw/day, the highest dose tested.

Bronopol in acidified water (pH 4) was given orally by gavage to mated New Zealand White rabbits at doses of 0, 5, 20, 40, and 80 mg/kg bw/day during day 7 to 19 of gestation ([redacted] 1991; A6.08.1_01). On day 28 of gestation the treated animals were sacrificed and dams and pups were examined for developmental toxicity. The dose levels were selected on the basis of the results of a range-finding study where signs indicative of maternal toxicity were seen in females treated with ≥80 mg/kg bw/day of Bronopol; these signs mainly consisted of loss in body weight gain, decrease in food consumption, and development of haemorrhages and ulceration in the gastric mucosa, as revealed by necropsy ([redacted] 1991; A6.08.1_04). In the main study maternal toxicity was observed in the high dose group only and consisted in reduced food consumption and decreased body weight gain. Developmental toxicity in the pups was confined to the high dose group where maternal toxicity was observed and consisted in increased reduced fetal body weight in both sexes and increased incidences of abnormalities indicative of general retardation of skeletal ossification and growth. The LOAEL for both maternal toxicity and developmental toxicity in the rabbit is 80 mg/kg bw/day. The NOAELs for both maternal toxicity and for developmental toxicity in the rabbit is 40 mg/kg bw/day.

Moreover, a guideline-compliant developmental toxicity study following OECD TG 414 in rabbits was conducted with Bronopol at dose levels of 0, 3, 10 and 30 mg/kg bw/day in acidified water (pH 4) ([redacted] 2006; A6_08_1-1). In the rabbit developmental toxicity study, following treatment during gestation day 7-27, maternal toxicity was seen at the highest dose level (30 mg/kg bw/day) characterised by clinical signs (decreased/absent faeces, noisy/labored respiration) and reduced body weight development and food consumption. Slightly increased incidences of some visceral (limited to a single litter) and skeletal malformations were observed in fetuses at the highest dose level. While the visceral findings were considered to be incidental, a relation to treatment of the skeletal findings was not excluded as some of them slightly exceeded the historical control ranges. However, the foetal findings were considered to be due to maternal toxicity (reduced food consumption/body weight loss especially marked in 3/4 litters with skeletal malformations). Conclusively, the NOAELs for both maternal toxicity and for developmental toxicity in the rabbit is 10 mg/kg bw/day.

This is in agreement with the findings of the rabbit developmental toxicity study conducted by [redacted] 1991 (A6.08.1_01), where also marginally increased incidences of malformations were seen at dose levels that induced marked maternal toxicity (higher NOAELs in comparison to the study by [redacted] 2006 (A6_08_1-1) may be due to the shorter treatment period).

A.3.10.2.2 Comparison with the CLP criteria

Reliable developmental toxicity studies demonstrated effects of Bronopol on developmental parameters in the presence of systemic/maternal toxicity, whereas no relevant adverse effects on developmental parameters were identified at lower dose levels in the absence of general toxicity. Thus, the available studies clearly indicate that adverse effects observed on prenatal development are linked to significant parental systemic toxicity.

Conclusively, these data clearly indicate that Bronopol is no developmental toxicant. Therefore, classification and labelling for developmental toxicity should not be required.

A.3.10.2.3 Overall conclusion on effects on development related to risk assessment

Not applicable for the CLH report.

A.3.10.3 Effects on or via lactation

There is no data available with respect to effects on or via lactation. According to toxicokinetic studies, bronopol does not appear to accumulate in breast tissue and, therefore, the possibility of its excretion via breast milk is low. In addition, postnatal toxicity results show that the substance was also not toxic to offspring.

A.3.10.4 Conclusion on classification and labelling for reproductive toxicity

Based on the available data and in the absence of effectos for the sexual function and fertility and the development, Bronopol is considered to be non-reprotoxic. Therefore, classification and labelling for reproductive toxicity should not be required.

A.3.10.5 Overall conclusion on reproductive toxicity related to risk assessment

Not applicable for the CLH report.

A.3.11 Aspiration hazard

No data exist which provide any reliable and good quality evidence that Bronopol poses any aspiration hazard.

A.3.12 Neurotoxicity

Neurotoxic potential of Bronopol has not been assessed in a study specifically designed for this purpose and no human data addressing neurotoxicity is available.

Data waiving	
Information requirement	Neurotoxicity (Annex II, Title 1, 8.13.2 (ADS)).
Justification	Neurotoxic potential of Bronopol has not been assessed in a study specifically designed for this purpose. The observed effects in repeated dose toxicity studies addressing neurotoxic endpoints are not clearly indicative of specific neurotoxicity or do not follow a particular pattern among different doses, species, sexes and routes of exposure. The characteristics and intended use of Bronopol do not require additional studies on neurotoxicity. Potential neurotoxic effects are expected to be covered by derived reference values for local and systemic effects. As neurotoxicity is not part of the core data set, generation of new test data is not required.

A.3.13 Immunotoxicity

Immunotoxicity of Bronopol has not been assessed in a study specifically designed for this purpose and no human data addressing immunotoxicity is available.

Data waiving	
Information requirement	Immunotoxicity (Annex II, Title 1, 8.13.4 (ADS)).
Justification	Immunotoxicity of Bronopol has not been assessed in a study specifically designed for this purpose. The observed effects in repeated dose toxicity studies are not indicative of immunotoxicity or do not follow a particular pattern among different doses, species, sexes and routes of exposure. The characteristics and intended use of Bronopol do not require additional studies on immunotoxicity. Potential immunotoxic effects are expected to be covered by derived reference values for local and systemic effects. As immunotoxicity is not part of the core data set, generation of new test data is not required.

A.3.15 Further Human data

No further human data are available for Bronopol.

A.3.16 Other data

Table A-37: Summary table of other data

Type of data / report, Reliability, Key / supportive study	Test substance (including purity), Vehicle	Relevant information about the study	Main effects, Observations	Reference
Mechanistic study **Rel. 2** **Supportive**	Bronopol (99.7% (0.10% sulphated ash, 0.06% water)) in lymphocyte culture medium (50 µL of a 3 mg/mL aq. solution of Bronopol was added to 5 mL of lymphocyte culture medium)	Bronopol decomposition and formaldehyde release in lymphocyte culture medium analysed using high pressure liquid chromatography and an ultraviolet spectrophotometer respectively.	The results are indicative of a rapid and extensive decomposition of Bronopol in chromosome medium 1A, with only about 10% of the initial concentration of parent compound remaining after 2 and 24 h of incubation at 37 °C. Also, at an initial concentration of 30 µg/mL Bronopol, a maximum concentration of 4.2 µg/mL formaldehyde was detected in the test medium after 2 h following addition of Bronopol to the chromosome medium 1A. Thereafter, the concentration of formaldehyde tended to slightly decrease.	[redacted] 1986 A6.10_01_a A6.10_01_b
Mechanistic study **Rel. 2** **Supportive**	Bronopol, in gelatine capsules	Minimal-disease cats received single oral doses of test material, owing to the known increased susceptibility of cats to methaemoglobinaemia. The dosage of Bronopol was increased each week until repeated vomiting occurred, to ensure that the highest tolerated level was administered	Vomiting was reported as only sign of toxicity. The female treated with 15 mg/kg bw, the male treated with 20 mg/kg bw and both animals treated with 25 mg/kg bw suffered from vomiting from about 10 min following dosing up to 24 h. No significant increase in methaemoglobin concentration could be evidenced in the blood samples of the treated cats 24 h after dosing. Blood samples of the cats treated with acetanilide (positive control) clearly was increased. Conclusively, Bronopol at doses up to 25 mg/kg bw, which was found to be toxic for cats, did not induce methaemoglobinaemia in this species.	[redacted] 1973 A6.10_02

A.4 Environmental effects assessment

A.4.1 Fate and distribution in the environment

There are several tests to study the degradation of Bronopol. It rapidly hydrolyses at pH 7, and several metabolites are formed from a series of possible reactions.

The analytical identification during hydrolysis studies has led to concentrations of some identified and unidentified metabolites higher than 10%. Same occurs in biodegradation test in the STP, where the parent disappears rapidly, and other metabolites are being formed leading to maximum degradations of around 55%.

Bronopol rapidly degrades in natural waters by abiotic degradation as well as biotically, but it is not considered as readily biodegradable. Further, some transformation products are formed abiotically (mainly 2-bromo-2-nitroethanol (BNE) as transient product via reversible reactions) and in the STP simulation study biotic process (mainly Tris(hydroxymethyl)nitromethane (TNM)).

This is a summary on relevant metabolites/degradants detected in studies:

Metabolite / degradant / transformation or reaction product	Compartment	% Active Substance
2-bromo-2-nitroethanol (BNE)	Freshwater	n.d.
Tris(hydroxymethyl)nitromethane (TNM)	Freshwater	n.d.
Bromonitromethane (BNM)	Freshwater	n.d.
2-bromoethanol (2BE)	Freshwater	n.d.
Nitromethane	Freshwater	n.d.

n.d. = not determined

Formaldehyde is one of the metabolites being released from the hydrolyzation of Broponol, but this a.s. has not been categorised as other preservatives that are usually referred to as “Formaldehyde releasers". This is because the mechanism of action of these formaldehyde releasers is known to rely, to a large extent, on Formaldehyde release, which is not the case for Bronopol, with its own mode of action.

Even though the release of formaldehyde has not been quantified in any of the test of this dossier, it is expected that formaldehyde is being released at some point and its toxicity could be part of the combined toxicity of Bronopol and degradation products.

A.4.1.1 Degradation

A.4.1.1.1 Abiotic degradation

Hydrolysis – Bronopol (parent compound)

Table A-38: Summary table- Hydrolysis

Method, Guideline, GLP status, Reliability, Key / supportive study	pH	Temp. [°C]	Initial TS concentration, C_0 [mol/L]	Half-life, DT50 [d]	Coefficient of correlation, r^2	Remarks Reaction rate constant, k_h [s^{-1}]	Reference
OECD 111, GLP, Rel. 2, Key	4	50	$3.0*10^{-5}$	13	0.9951	0.0538	[redacted] 2003 (A7_1_1_1_1-01)
	4	60	$3.0*10^{-5}$	3	0.9901	0.235	
	4	70	$3.0*10^{-5}$	0.7	0.9994	1.00	
	4	25	-	100[a]	-		
	7	50	$3.0*10^{-5}$	< 2 h[b]	-	-	
	9	50	$3.0*10^{-5}$	< 2 h[b]	-	-	
Directive 92/69/EG, C.7, GLP, Rel. 3	4	50	No data	20.7	0.976	3.871×10^{-7}	[redacted] 2000 (A7_1_1_1
	4	70	No data	1.1	0.998	7.539×10^{-6}	
	4	80	No data	0.26	0.999	3.105×10^{-5}	

							_1-02)
	7	50	No data	< 0.7 h[b]	-		
	9	50	No data	< 0.1 h[b]	-	-	
OECD 111, preliminary test, no GLP, Rel. 2, key	4	50	2, 10, 25, 100 and 1000 ppm	>1 d and <1 y (25°C)	No data	No data	[redacted] 1996 (A7.1.1.1.1 _01)
	7, 9	50	10, 25, 100 and 1000 ppm	<2.4 h (25°C)	No data	No data	
Publication, complementary data Rel. 3 (supporting)						Information on Bronopol degradation products and - pathways in water	[redacted] 1991 (A7.1.1.1.1 _02)

[a] Reaction rate extrapolated by means of the Arrhenius equation from ENV TAB 182
[b] Half-life estimated from preliminary test

In the first (key) study ([redacted] 2003; A7_1_1_1_1-01), the hydrolysis of Bronopol was rapid at elevated temperature (50°C) in neutral to basic media (pH 7 and 9, respectively). Hydrolysis half-lives were <2 hours at 50ºC in the tier 1. At 2 hours almost no Bronopol was remaining. In the acidic (pH 4), the temperature dependence of the hydrolysis rate constant was determined on a tier 2.Thus, a half-life of around 100 days was estimated for 25°C (approximately 334 days at 12ºC), indicating that Bronopol is stable to hydrolysis under acidic conditions (pH 4). The half-life at pH 7 and 12°C was around 2 days, estimated from the preliminary test by Arrhenius equation according to ENV TAB entry 182 (this is not an accurate transformation as it has been done from 50 ºC, which is out of the scope of Arrhenius equation as in ENV TAB 182). It was seen during this study that several metabolites were formed from the hydrolysis of Bronopol. According to the results, product E and product A were the most important peaks of the HPLC. Product E was identified as **2-Bromo-2-Nitroethanol** (BNE) formed in all pH, at percentages ranging from 8 to 16% in the preliminary test (tier 1) and reaching up to 44.2% in the tier 2 test (only accomplished at pH 4). In tier 1, at pH 9 it was seen that the concentration of BNE diminished after 24 hours. The proportion of product A in the preliminary test ranges from 18.2% at pH 4 to 77.3% at pH 9. No test shows diminishment of product A at any time, temperature or pH. According to OECD TG 111, the higher tier test should be performed at the pH values at which the test substance was found unstable as defined by the preliminary test, in this case, pH 7 and 9, instead of at pH 4. Nevertheless, the hydrolysis products at pH 4, 7 and 9 are the same (the chromatograms support this) following the same degradation pathway: same metabolites generated, being BNE identified as major hydrolysis product and intermediate in several proposed degradation pathways; the identified as product A could be a mixture of very polar substances like Tris(hydroxymethyl)nitromethane and Formaldehyde. Other metabolites appeared below 10%.

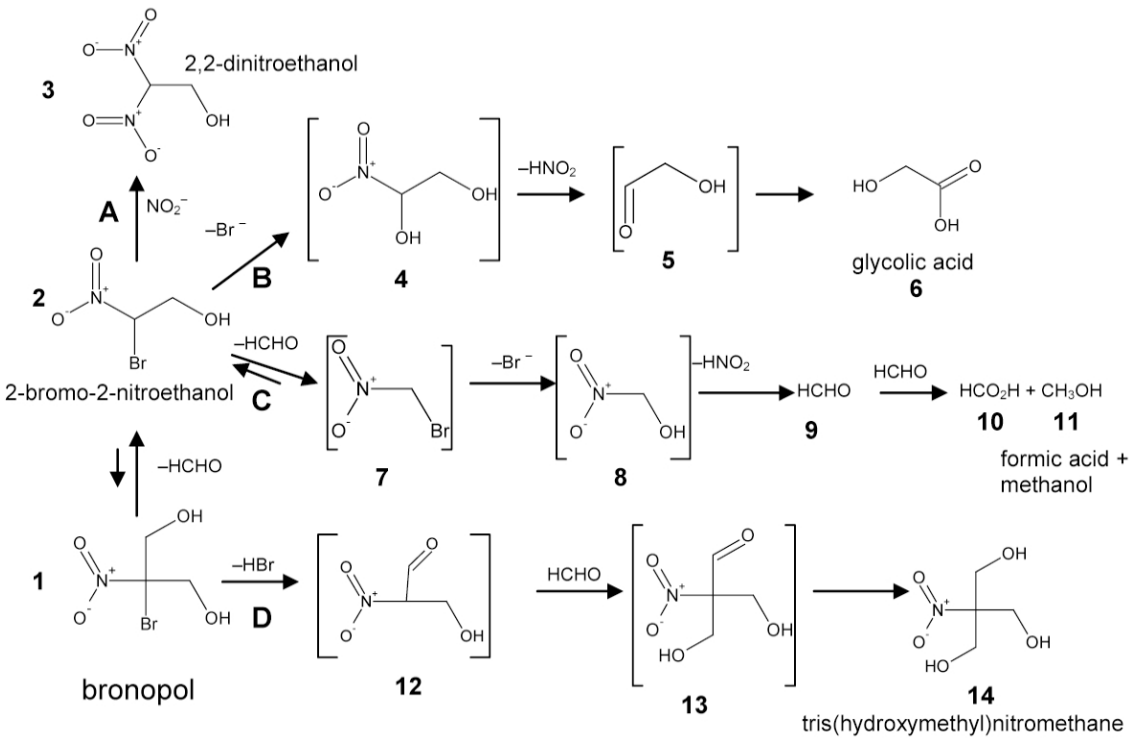
The second hydrolysis study ([redacted] 2000; A7_1_1_1_1-02) is not well documented. The tier 2 has been also performed t pH4 instead of at unstable pHs (7-9) and the methodology hasn't been described, the study only reports the final values of the experiment. Hence, the study is considered as supporting information. Half-lives are nevertheless comparable to those obtained in the study by [redacted] (2003; A7_1_1_1_1-01).

In the third (key, non-GLP) study ([redacted] 1996; A7.1.1.1.1_01), hydrolysis of Bronopol was investigated according to OECD TG 111 (tier 1 test) at pH 4, 7 and 9 at 50°C in sterile buffer solutions. Rapid hydrolysis of Bronopol was both pH and concentration dependent. At the end of the test after 5 to 7 days, Bronopol showed a degradation of 25 to 55.6% at pH 4, 72.5 to 91.8% at pH 7 and 91.1 to 100% at pH 9. The hydrolysis rate increased whilst substance concentration decreased. At pH 7, half-life of 0.0245 days was derived by using CAKE 3.4 software at 50°C (r^2 = 0.9474) referring to test concentration of 25 ppm, which can be considered as representative as a worst case of the uses of Bronopol where hydrolisation is taking place. At pH 4 and 25°C, $t_{1/2}$ was between > 1 day and < 1 year. The results of the study revealed that Bronopol underwent hydrolysis with a significant pH

dependency. At pH 7 and 9, the rate of hydrolysis was rapid, but also showed a distinct concentration dependency with the rate of hydrolysis increasing as concentrations decreased. At pH 4, the Bronopol molecule was intrinsically more stable, but results showed a steady hydrolysis with time and a similar though less marked concentration effect.

In addition to those standard tests, a publication is available by [redacted] (1991; A7.1.1.1.1_02). The authors used NMR and HPLC to characterise and identify various decomposition products of Bronopol in concentrated KOH solutions (pH > 14). The conditions of those experiments bear no relevance to conditions in the STP or the environment. The reaction pathways inferred in the publication are therefore not necessarily relevant for degradation processes assessed in this dossier. However, [redacted] (1991; A7.1.1.1.1_02) confirmed the fact that the abiotic degradation of Bronopol accelerates with increasing pH.

In water and at environmental relevant pH values, hydrolysis of Bronopol takes place very rapidly, with a significant pH and concentration dependency displaying an accelerated rate of hydrolysis at lower concentrations and elevated pH. Decomposition of Bronopol in water results in the formation of **Tris(hydroxymethyl)nitromethane** (TNM), **Glycolic acid**, **Formic acid**, **Methanol** (all <5%, 24 h) and **2,2-Dinitroethanol** (<1%). Four concurrent degradation pathways resulting in the production of these degradation products have to be considered, with 3 of them involving BNE which is formed from Bronopol and increases in concentration over about 30 min (pH 9, 25°C) following pseudo first-order kinetics. Thereafter, the concentration of BNE remained constant in equilibrium with the parent compound Bronopol.

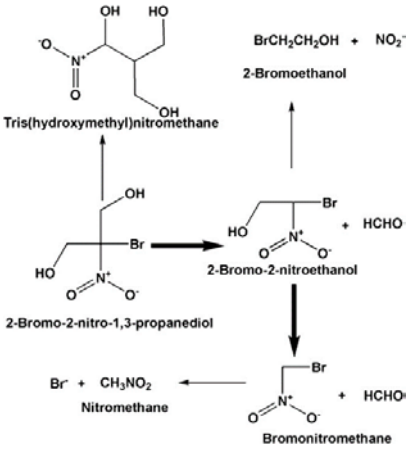


Main degradation pathways described by [redacted] (1991; ; A7.1.1.1.1_02). Reactive intermediates are shown in brackets. Identified degradation products from Bronopol (1) were 2-Bromo-2-Nitroethanol (2), 2,2-Dinitroethanol (3), Glycolic acid (6), Formic acid (10) and Methanol (11), and Tris(hydroxymethyl)nitromethane (14).

Aside from the above-mentioned substances, **Bromonitromethane** (BNM) additionally occurs in literature as degradation product of Bronopol (Cui 2011, Wang 2002, Ostrovskaya 2000). According to Wang (2002), BNE and BNM and a small amount of 2-Bromoethanol

were produced from degradation of Bronopol in aqueous solutions at 40°C. The concentration of BNE decreased with time and it was anticipated that it was further degraded either to BNM via release of formaldehyde or to 2-Bromoethanol via the release of a nitrite ion as a reactive intermediate. However, this process was found to be very limited in aqueous solution, especially at ambient temperature. This thesis is supported by the observations in Ostrovskaya (2000) where BNE was identified as one of the major decomposition products of Bronopol and two other peaks were identified over time, BNM and 1-bromo-1-nitroethene. These secondary peaks occurred as a result of further decomposition of the intermediate BNE.

Additionally, Cui *et al*. (2010) mentioned that in industrial products and aqueous solutions, bronopol rapidly degrades to various transformation products, consisting of 2-bromo-2-nitro-ethanol (BNE), bromonitromethane (BNM), tri(hydroxymethyl)nitromethane (TNM), nitromethane (NM), 2-bromoethanol (2-BE), formaldehyde (FA), and other unidentified chemicals. 2-bromo-2-nitro-ethanol is a reactive intermediate in three of the four degradation pathways identified by Challis and Yousaf (1991). Even in the industrial products (Wang *et al*., 2002), 2-bromo-2-nitro-ethanol first forms from hydrolysis of Bronopol with the release of formaldehyde, and further transforms to bromonitromethane and 2-bromoethanol, though BNE can also react with formaldehyde to produce the parent again. These results indicate that BNE is a major intermediate of bronopol in the environment as well as in the industrial products and industrial water systems. 2-bromo-2-nitro-ethanol degraded to acid formic and methanol, where formaldehyde is an intermediate product. Cui *et al*. also performed hydrolysis and photolysis experiments in natural waters giving hydrolysis rates as well as the proposed degradation pathway:



Hydrolysis kinetics of bronopol (BNP) and 2-bromo-2-nitroethanol (BNE) in natural waters ($R > 0.95$, $P < 0.0001$).

BNP						BNE[a]	
Buffer solution	K_T (d^{-1})	$t_{1/2}$ (d)	Water sample	K_T (d^{-1})	$t_{1/2}$ (d)	K_T (d^{-1})	$t_{1/2}$(d)
pH 4.0	–[b]	–[b]	1	7.375	0.094	0.0280	24.750
pH 6.7	0.136	5.089	2	6.431	0.108	0.0543	12.767
pH 7.6	1.047	0.662	3	5.939	0.117	0.0626	11.063
pH 9.0	8.599	0.081	4	4.829	0.144	0.0183	37.848
pH 10.0	42.964	0.016	5	5.306	0.131	0.0135	51.448

[a] Represents degradation rates of BNE after reaching maximum concentrations, based on the suppose that degradation of the compound was ignored before reaching maximum concentrations.
[b] Represents no calculation.

According to these data from the available literature, hydrolysis DT50 would be around 0.1 d in natural waters at typical T and pH for BNP. This supports the results from the studies presented, which is less than 2 hours at 50ºC and pH 7 and less than half day at natural water temperature.

According to Ostrovskaya (2000), the whole degradation process of Bronopol can be summed up as follows: Bronopol is degraded to BNE by losing a formaldehyde molecule and BNE further degrades following two pathways: 1) loss of an additional formaldehyde molecule becoming BNM or 2) loss of the hydroxyl group with the formation of a double bond between carbons becoming 1-bromo-1-nitroethane. The decomposition of BNE occurs at a much slower rate than the parent compound Bronopol.

Formaldehyde plays an important role in the degradation of Bronopol as it is involved in many processes as displayed in the scheme by [redacted] (1991; A7.1.1.1.1_02). Bryce (1978) stated that a number of reactions involving formaldehyde occur simultaneously. The

overall result is that the formaldehyde concentration tends to a maximum which is lower than an equimolar ratio. Kajimura (2008) investigated the release of formaldehyde upon the decomposition of Bronopol. One of the results indicated that the release of formaldehyde upon the decomposition of Bronopol was dependant on temperature reaching up to 50% of the parent compound incubated at 60°C for 90 minutes. When Bronopol solution was prepared with weakly alkaline buffer (pH 8) and stored for 24 hrs, the concentration of formaldehyde reached 30% of the parent compound. In contrast, under acidic conditions (pH 2) little formaldehyde was produced over 50 days.

Hydrolysis – Tris(hydroxymethyl)nitromethane and 2-bromo-2-nitroethanol (metabolites)

Table A-39: Summary table- Hydrolysis

Method, Guideline, GLP status, Reliability, Key/supportive study	pH	Temp. [°C]	Initial TS concentration, C_0 [mol/L]	Half-life, DT50 [d]	Coefficient of correlation, r^2	Remarks Reaction rate constant, k_h [s^{-1}]	Reference
Publication, complementary data (supporting)	5, 7, 9	25	200 ppm	3.4 d	No data	Degradation of Tris(hydroxymethyl) nitromethane by Hydrolysis	[redacted] 1993 (A7.1.1.1.1_04)
OECD 111, GLP, Rel. 2	4	50	$3.0*10^{-5}$	stable	Not applicable for Tier 1 test	-	[redacted] 2012 (A7_1_1_1_1-05)
	7	50	$3.0*10^{-5}$	0.91	Not applicable for Tier 1 test	-	
	9	50	$3.0*10^{-5}$	0.67	Not applicable for Tier 1 test	-	

TRIS NITRO did not degrade at pH 5. At pH 7 a half-life of 3.42 days was determined and at pH 9 the half-life was 2.43 days. However, the presence of formaldehyde in closed vials was shown to stabilize TRIS NITRO. This was expected based on the fact that TRIS NITRO is synthesized by a reversible reaction of three moles of formaldehyde with one mole of nitromethane.

2-bromo-2-nitroethanol exhibited increasing hydrolysis rates with increasing pH. After five days at 50°C, degradation of the test substance was less than 3% at pH 4, 76% at pH 7, and 94% at pH 9. Since less than 10% degradation occurred after 5 days at pH 4, the test substance is considered hydrolytically stable at pH 4. A tier 2 has not been provided. The DT50 at 12ºC and pH 7 was extrapolated from DT50 at 50ºC using Arrhenius equation (TAB ENV 182) leading to a value of around 23 days. This value is just an approximation, as the scope of the equation in TAB ENV 182 is from 0 to 30ºC. Nevertheless, data from literature shows a hydrolysis DT50 of around 11-12 d in natural waters at pH 7 (Cui *et al*. 2011), which fits with the test results (0.91 days at 50ºC = 11 days at 20ºC = 23 days at 12ºC).

Compared to BNP, degraded within a few hours at 25ºC, the hydrolytic half-life of BNE in natural waters is relatively high (Cui *et al*. 2011). The half-life varied due to the different pH values and water samples. 2-Bromo-2-nitroethanol (BNE) was catalytically degraded by natural waters, though this metabolite is hydrolytically more stable than parent (Cui *et al*. 2011).

Phototransformation in water

Table A-40: Summary table- Photolysis in water

Method, Guideline, GLP status, Reliability, Key/supportive study	Initial molar TS concentration	Total recovery of test substance [% of appl. AS]	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (Φ^c_E)	Half-life ($t_{1/2E}$)	Remarks	Reference
OECD draft (Aug. 2000), US EPA OPP 161-2 (1982), EPA 540/9-90-078 (1989), GLP, Rel. 2	5 mg/L ($2.3*10^{-5}$ mol/L)	Day 0: 100.0 Day 16: 33.0	Not determined	0.08192 day^{-1}	0.00782 molecules $photon^{-1}$	30-40°N: 20 d 50°N: 21 d (Simple first-order half-life for midsummer sunlight days, average daylength for irradiation 75% of 12 hours = 9 hours)	mean radiant flux incident on the receiving surface (irradiance) in the range of 300-400 nm: 56.2 W/m²	[redacted] 2007 (A7_1_1_1_2-01)
US EPA OPP 161-2, GLP, Rel. 2	5 µg/g	54.35 (24 h) 12.62 (72 h) 0 (168 h)	Not applicable	Not applicable	Not applicable	24.3 h		[redacted] 1992 (A7.1.1.1.2_01)

Bronopol was shown to be susceptible to photolysis in water, with half-lives of 20-21 days for midsummer sunlight days of average length (9 hours sunlight) at latitudes of 30-50°N ([redacted] 2007; A7_1_1_1_2-01). The mean radiant flux incident on the receiving surface (irradiance) in the range of 300-400 nm was 56.2 W/m², thus slightly higher than the light intensity of natural midsummer daylight with vertical incidence of the sun on a clear, cloudless day (43.7 W/m², August 31, 2006 in Itingen, Switzerland). The study was conducted in a pH 5 buffer solution at 25°C, because Bronopol undergoes hydrolysis at higher pH values. Since non-radiolabelled Bronopol was used, no degradation products were characterised.

In the second study ([redacted] 1992; A7.1.1.1.2_01) with radiolabelled test material [^{14}C]-Bronopol in buffered aqueous solution (pH 4) was photodegraded under continuous artificial sunlight at 25°C with a degradation half-life of ca. 24 h, equivalent to 2 days under natural sunlight conditions assuming 12 h of sunlight at an average intensity equal to that used in the study. No photodegradation was observed at darkness. At least 3 degradation products were observed, however, only 2 were tentatively identified as **tris-(hydroxymethyl)-nitromethane** and **carbon dioxide**. Tris-(hydroxymethyl)-nitromethane could be shown to undergo further photodegradation between 72 and 168 h.

Under natural conditions, hydrolysis and primary biodegradation in water are expected to be more rapid than photolysis of Bronopol. This is especially true taking into account that sunlight penetrates only the very uppermost layers of natural waters. Therefore, potential phototransformation products of Bronopol are expected to occur only at negligible levels in the environment.

Bronopol has a very low Henry´s Law constant of $1.16*10^{-6}$ Pa*m^3/mol at 25°C (calculated with EPI suite 4.1.1) and therefore volatilisation is not to be expected.

Estimated photo-oxidation in air

Table A-41: Summary table- Photo-oxidation in air

Model	Light protection (yes/no)	Estimated daily (24h) OH concentration [OH-radicals/cm³]	Overall OH rate constant [cm³/molecule sec^{-1}]	Half-life [hr/d]	Reference
Estimation by AOPWIN 3.1 software, v1.70	Not applicable	$0.5*10^6$ (global annual average OH-radical concentration (BUA, 1992) referenced in TGD Part II[2])	Rate constant for reaction with OH-radicals (k_{OH}): $1.325*10^{-12}$ Rate constant for reaction with ozone (k_{OH}): *No reaction, since Bronopol contains no unsaturated carbon-carbon bonds*	Based on 12 h sunlight: 24.2 days Based on 24 h sunlight: 12.1 days	[redacted] 2007 (A7_3_1-01)
Estimation by AOPWIN software, v1.91	Not applicable	$1.5*10^6$	$1.33*10^{-12}$	Based on 24 h sunlight: 12.106 days	[redacted] 2005 (A7.3.1_01)
Estimation by AOPWIN software, v1.91	Not applicable	$0.5*10^6$ OH-radical concentration	2-Bromo-2-Nitroethanol 0.957E-012 Trishydroxymethylnitromethane 1.923E-012 Formaldehyde 8.13E-012	2-Bromo-2-Nitroethanol Half-life: 16.8 days. Trishydroxymethyl nitromethane Half-life: 8.34 days. Formaldehyde Half-life: 1.97 days.	[redacted] 2005 (A7.3.1_02)

The rate constant for phototransformation of Bronopol in air was estimated using the AOPWIN software. The Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The program is based on a quantitative structure analysis developed by Atkinson.

A tropospheric half-life of 24.2 days was calculated for reaction of OH-radicals with Bronopol, assuming 12 h of sunlight, 25°C, and an OH-radical concentration of $0.5*10^6$ cm^{-3} ([redacted], 2007; A7_3_1-01). Using a 24-hours day and a mean daily OH concentration in air of $1.5*10^6$ OH-radicals per cm³, a half-life in air of 12.106 days was assessed (overall OH rate constant: 1.33 10^{-12} cm³/molecule*sec). Based on these estimations, Bronopol will be rather slowly degraded in air.

[2] Technical Guidance Document on risk assessment 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, Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II. April 2003. Catalogue number LB-NC-20418-EN-C.

A.4.1.1.2 Biotic degradation, initial studies

Biodegradability (ready/inherent)

Table A-42: Summary table - biodegradation studies (ready/inherent)

Method, Guideline, GLP status, Reliability, Key/supportive study	Test type[1]	Test parameter	Inoculum			Additional substrate	Test substance concentration	Degradation		Remarks [positive control]	Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]		
OECD 301B (modified), GLP, Rel. 2 Key	Ready	$^{14}CO_2$ evolution	Activated sludge from municipal sewage	30 mg/L suspended solids	No	No	0.05 and 0.5 mg/L ^{14}C-Bronopol	28 days	57 and 51 Abiotic degradation: 13 and 10	Positive control (sodium benzoate) and toxicity control: 99% degradation after 14 days	[redacted] 2002a (A7_1_1_2_1-01)
OECD 301B, modified procedure, GLP, Rel. 2	Ready	$^{14}CO_2$ release, biomass	Activated sludge	30 mg/L (d.w.)	No	No	0.1 mg/L ^{14}C-Bronopol	29 days	About 90 at test end, but only 50 at the end of 10d-window	Positive control ([2-^{14}C]-Acetic acid, sodium salt)	[redacted] 1999 (A7.1.1.2.1_01)
Directive 92/69/EEC Method C.4-B (modified), comp. to OECD 301E, GLP, Rel. 3 (supporting information)	Ready	Primary bio-degradation	Activated sludge from municipal sewage	0.5 mL/L (effluent in reaction mixture)	No	No	1 mg/L Bronopol	28 days	0 disappearance (100% within 7 days) not caused by microorganisms	Positive control (sodium benzoate): 98% degradation after 14 days	[redacted] 2001 (A7_1_1_2_1-02)
OECD 301B (modified), Com. Reg. (EC) No 440/2008 Method C.4-C, GLP, Rel. 2	Ready	$^{14}CO_2$ evolution	Activated sludge	30 mg/L (d.w.)	No	No	30 µg/L ^{14}C-Bronopol	28 days	About 20% at test end, and 16 at the end of 10d-window Abiotic degradation: 3	Positive control (Benzoic acid, sodium salt, [ring-14C(U)]-): 70% degradation after 14 days	[redacted] 2022 (A7.1.1.2.1_02)
OECD 304A, 302B, modified procedures, GLP, Rel. 3	Inherent	CO_2 release, biomass	Activated sludge	250 mg/L (d.w.)	No	No	1 mg/mL Bronopol	64 days	100	Positive control (D[U-^{14}C]-Glucose): 67% degradation after 14 days	[redacted] 1994 (A7.1.1.2.2_01)
Studies on metabolites											
OECD 301F (1996), GLP, Rel. 2	Ready	O_2 consumption	Activated sludge	30 mg/L (suspended solids)	no	no	19 and 46 mg/L Tris-hydroxymethyl-nitromethane (Tris Nitro, TNM)	28 days	13.4 at test end in 46 mg/L treatment, 8.9 at test end in 10 mg/L treatment	Positive control (sodium benzoate): >60% degradation after 3.3 days Inhibitory effects	[redacted] 2002b (A7_1_1_2_1-05)

Method, Guideline, GLP status, Reliability, Key/supportive study	Test type[1]	Test parameter	Inoculum			Additional substrate	Test substance concentration	Degradation		Remarks [positive control]	Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]		
									Abiotic control: no degradation based on O_2 consumption and CO_2 production	observed in toxicity control, although criteria for inhibition by test substance was not met (73% O_2 consumption instead of <25% after 14 days)	
OECD 301F (1992), Com. Reg. (EC) No 440/2008 Method C.4-D, GLP, Rel. 4	ready	O_2 consumption, CO_2 evolution, and DOC removal	Activated sludge	30 mg/L (dry solids)	no	no	332 mg/L 2-bromo-2-nitroethanol (BNE), equivalent to 31.2 mg/L ThOD	28 days	No biodegradation observed due to microbial inhibition	Positive control (aniline): >60% degradation after 4.8 days No biodegradation in toxicity control which confirms the inhibitory effect of the the test substance at the concentration tested.	[redacted] 2012 (A7_1_1_2_1-07)
Screening test, modified OECD 301B (1993), non-GLP, Rel. 3	ready	$^{14}CO_2$ evolution	Activated sludge	30 mg/L (dry solids)	no	no	20 and 100 µg/L ^{14}C-2-Bromo-2-nitroethanol	29 days	At test end: 100 in 20 µg/L treatment, 95 in 100 µg/L treatment At the end of 10d-window: >60% in both treatments	Screening test, no positive control, blank control or abiotic control were prepared	[redacted] 2021 (A7.1.1.2.1_03)

[1] *Test on inherent or ready biodegradability according to OECD criteria*

In the modified OECD 301B test ([redacted], 2002a; A7_1_1_2_1-01), considerable mineralisation of Bronopol of 51% (at 0.5 mg/L) and 57% (at 0.05 mg/L) were observed within the 28-day test period. Accordingly, the classification target of 60% mineralisation is not met and Bronopol can thus not be classified as readily biodegradable. However, primary biodegradation was substantial for Bronopol, with no detectable concentrations of the active substance already after the shortest sampling interval of one day. Biologically mediated degradation was the major degradation pathway of Bronopol in activated sludge, although considerable abiotic degradation was also evident. Consequently, in the test substance reaction mixtures a large fraction of radioactivity was recovered as $^{14}CO_2$ and only 27-35% of applied radioactivity (AR) remained in solution. In contrast, 75% of AR remained in solution in the abiotic control and only 10-13% of AR were recovered as $^{14}CO_2$. Since rapid degradation occurred via both biotic and abiotic pathways it can be concluded that Bronopol will not persist in the environment.

A further test on ready biodegradability was performed, which followed a modified procedure based on OECD TG 301B ([redacted], 1999; A7.1.1.2.1_01). ^{14}C-labelled Bronopol was used and in deviation from the guideline, the methods were modified with respect to the test substance concentration, incubation vessels and method of determining the test substance mineralisation. In fact, the test substance concentration of 0.1 mg/L was chosen to avoid bacteria toxicity; the yield of CO_2 derived from the test substance was measured as mineralisation to $^{14}CO_2$ by liquid scintillation counting (LSC), and ^{14}C-material present in the cells was determined by means of combustion analysis using a biological oxidiser (Packard System 387 sample oxidiser). Two experiments were undertaken.

In experiment 1, around 10% of mineralisation was reported for day 1 whereas 55% mineralisation was achieved within the 10-day-window, and approximately 89% after a period of 29 days. In experiment 2, around 10% degradation appeared within the first two days, followed by 45% degradation during the 10-day-window. After 29 days, 67% mineralisation was achieved. For the radioactivity related to cell biomass, a mean value of 12% was found to be associated with cell biomass by material balance measurements at day 29, bringing the total biodegradation in experiment 1 to 100% at day 29 and to about 80% for experiment 2. Considering the results of both experiments, a mean degradation rate of about 90% of the initial ^{14}C derived from the test substance Bronopol (0.1 mg/L) was shown to be biotransformed by day 29, and consisted of about 78% $^{14}CO_2$ and about 12% ^{14}C incorporated into microbial biomass.

The test from [redacted] (2001; A7_1_1_2_1-02) was not considered reliable. The study is valid only as support information because it has not enough quality. The explanations and details of procedures are weak. There is another test from the same author (2000), provided only as additional information. These two tests do not reveal information on readily biodegradation of Bronopol in sewage sludge, either because the Bronopol concentration was toxic to bacteria or because only primary degradation was followed with the shortest sampling interval being 7 days.

In 2022, the biodegradation behaviour of Bronopol was again investigated in a GLP study according to a modified procedure based on OECD TG 301B ([redacted] 2022; A7.1.1.2.1_02). Radiolabelled Bronopol was used as test substance at the concentration of 30 µg/L (calculated based on the amount of 2.74×10^7 dpm TAR (total applied radioactivity)) and a mixture of radiolabelled (15 µg/test vessel) and unlabelled sodium benzoate (equivalent to 5 mg TOC/L) was used as reference substance. The test concentration was proven to be non-inhibitory against the microorganisms by reaching 40% degradation in the inhibition control after 14 days and the result of the positive control (70% degradation after 14 days) confirmed the viability of the inoculum. After the exposure period of 28 days, 3% degradation was observed in the abiotic control and 20% degradation in the test mixtures with Bronopol missing the threshold for readily biodegradability (60% at the end of 10 day-window) as well as for ultimately biodegradability (60% at test end). However, a constant degradation of Bronopol was observed which had not reached a plateau by the end of the test.

Further information on the degradation of Bronopol in activated sludge was published by Ehresmann (1992). Fungi specialised on the co-metabolic degradation of Bronopol were isolated from activated sludge and incubated with carbon sources, mineral medium and Bronopol at various concentrations. Degradation of Bronopol was followed via AOX and Bromide concentrations. Bronopol was biodegraded by reductive halogenation to 1-nitro-1,2-propanediol, which was further shown to be rapidly biodegraded.

The specific fungi used in those experiments do not allow extrapolating the postulated degradation pathway to real STP or environmental conditions. However, the degradation rate of Bronopol was shown to increase with the amount of additional carbon sources added to the inoculum. This demonstrates that Bronopol degradation by specialised microorganisms under realistic conditions is more rapid than in the OECD 301 test protocols where Bronopol is the sole carbon source.

An inherent biodegradability test was also conducted according to modified procedures based on OECD TG 304A and 302B ([redacted], 1994; A7.1.1.2.2_01). During this test, Bronopol disappeared completely within 3 days. A major metabolite (similar to 2-nitropropane-1,3-diol) appeared around Day 3 which was again subjected to rapid and complete biodegradation (up to 100%) on Day 17. At the end of the test (on Day 64), biodegradation was quantified as 68 and 23 % related to the CO_2 evolution and biomass production, respectively. For the reference substance, D[U-^{14}C]-Glucose, a biodegradation of 46% was achieved within 3 days, and 78% at day 40. The study shows some inconsistencies. The raw data were not submitted after asking; hence, it was not possible to recalculate the percentage of biodegradation. Additionally, the calculation of the biodegradation summing the radiolabelled material found in cells is not specified in any guideline, so the procedure is not reliable. The C14 could be adhered to the cells or organic material rather than incorporated into their cell material. Further, no validity criteria were met: OECD 302B specifies that at least 70% of control substance must be degraded by day 14 of the test but this does not occur (the positive control using glucose showed a mineralisation of 67.45 % at day 14 of the test). In any case, analysing the results of the test, compound a total of 67.24 % of the 14C was converted to 14CO2. Therefore, the substance is not mineralised. There are major deficiencies if comparing to OECD test such as the amount of inoculum used. The quantification of metabolites and the % of them compared to the Bronopol is not explained. Additionally, the amount of test substance used is much higher than the EC20 for microorganisms. Due to all this, the study is considered as supporting information only.

Assessment of metabolites

In the study by [redacted] (2002a; A7_1_1_2_1-01), the use of radiolabelled active substance allowed the characterisation of **tris-(hydroxymethyl)-nitromethane** (TNM) as major degradation product. Its peak concentration was already reached at day 1 and amounted to 71-74% of AR. By the last sampling (28 days), its concentration had fallen below 50% of the peak concentration.

In a second study by [redacted] (2002b; A7_1_1_2_1-05), the biodegradation potential of TNM was directly evaluated using the manometric respirometry test (OECD 301F). After 28 days, biodegradation of the test material reached 13.4% at a maximum based on oxygen consumption used as the primary indicator of biodegradation. No biodegradation was observed in the abiotic control. Biodegradation in the toxicity control was delayed in comparison to the positive control indicating an inhibitory effect of the test substance. However, as the criteria for inhibition of the inoculum given by the guideline was not met, the poor biodegradation at the end of the test cannot solely be explained by inhibition of the inoculum. Since the threshold level for readily biodegradation (60% degradation at the end of 10-day window) was not reached, TNM cannot be classified as “readily biodegradable" according to OECD TG 301F and is consequently considered as not readily biodegradable in further evaluations.

The biodegradation behaviour of **2-bromo-2-nitroethanol** (BNE) as second major degradation product was also investigated in a study according to OECD TG 301F ([redacted] 2012; A7_1_1_2_1-07). The test substance in a concentration of 332 mg/L did not meet the criteria of readily biodegradable. However, lack of biodegradation of aniline in the toxicity control and cumulative oxygen consumption in the test mixtures that was less than the blank control indicated that the test substance was inhibitory to the microbial inoculum under the test conditions. Hence, this study was considered not reliable.

To be able to decrease the test concentration to a non-inhibitory level, a second study with radiolabelled BNE was conducted ([redacted] 2021; A7.1.1.2.1_03). In this screening test based on OECD TG 301B ^{14}C-BNE was added in two concentrations (20 µg/L and 100 µg/L, 2 replicates each) to an inoculum of activated sludge from a municipal wastewater treatment plant and incubated under aeration for 29 days at 22.0±0.5°C. Samples were taken on days 0, 6, 20, 29, and 30 to measure the evolved CO_2. Degradation was calculated based on the evolved CO_2 trapped in absorption solution (NaOH) by comparing the detected radioactivity

in the evolved CO_2 to the initial total applied radioactivity (TAR). The threshold of 60% degradation was passed after 5 days in the low concentration and after 9 days in the high concentration, respectively, but in both cases before the end of the 10-day window. The 10-day window was met in three replicates but in the second replicate for the lower concentration, it was not included in the final evaluation due to an irreparable leak in the absorption flask causing a loss of CO2 leading to an underestimation of the biodegradation of the test substance. It has also been observed that in the data of liquid scintillation counting of the absorption solution ($^{14}CO_2$) the values of the different replicates were very different. At the end of the exposure, the test substance was degraded to 95±5% in the higher test concentration and to 100% in the lower test concentration, so the pass levels for ready biodegradability were met. Nevertheless, this test can only be used as supporting information due to significant deviations between replicates and lack of controls (abiotic, inhibition and reference substance) in the test. In addition to the lack of information on preparation and conditions of the sample.

In addition to TNM, a group of polar degradation products of low molecular weight was characterised by [redacted] (2002a; A7_1_1_2_1-01). These were judged to consist mainly of **glycolic acid** and traces of **formaldehyde**. In the STP, those highly polar compounds will be readily biodegraded and will not partition from the water phase into sludge or air. Therefore, no relevant emissions of degradation products are to be expected from the STP.

Conclusion on biodegradation processes of Bronopol:

In water and at environmental relevant pH values, hydrolysis of Bronopol takes place very rapidly, with a significant pH and concentration dependency displaying an accelerated rate of hydrolysis at lower concentrations and elevated pH. Decomposition of Bronopol in water results in the formation of TNM, glycolic acid, formic acid, methanol (all <5%, 24 h) and 2,2-nitroethanol (<1%). Four concurrent degradation pathways resulting in the production of these degradation products have to be considered, with 3 of them involving BNE as reactive intermediate. It could be shown that at pH 9 and 25°C, BNE is formed from Bronopol and increases in concentration over about 30 min following pseudo first-order kinetics. Thereafter, the concentration of BNE remained constant in equilibrium with the parent compound Bronopol.

In water, Bronopol is photodegraded at environmentally relevant temperatures; a half-life of 24.3 h was reported at 25°C. TNM and CO_2 were tentatively identified as two of three degradation products, with TNM being further photodegraded.

Bronopol is slowly degraded in air by photochemical processes, and a half-life in air of 12.106 days was calculated for this substance.

Bronopol's potential of readily biodegradation was investigated in several studies according to different methods described in the OECD guideline 301. While the criteria for readily biodegradability was not fulfilled in neither of these studies, signs of abiotic degradation (e.g. hydrolysis) were observed indicating that abiotic degradation processes are predominant under the respective test conditions and thus at environmental relevant pH values. At higher test concentrations inhibition of the microbial inoculum was observed.

A.4.1.1.3 Rate and route of degradation including identification of metabolites and degradation products

A.4.1.1.3.1 Biological sewage treatment

Not applicable for the CLH report.

A.4.1.1.3.2 Biodegradation in freshwater

Aerobic aquatic degradation

No degradation study in freshwater has been performed, considering the existing hydrolysis and ready biodegradation studies with [^{14}C]Bronopol, together with literature data, which demonstrate that rapid primary degradation of environmentally realistic concentrations occurs. For instance, the ready biodegradation test for [^{14}C]Bronopol achieved 51 to 57% $^{14}CO_2$ production in 28 days, just below the 60% criteria for readily biodegradable. Primary biodegradation of the [^{14}C]Bronopol occurred within 1 day in the viable mixtures, and within 3 days in the biologically inhibited controls.

Further, considering the data from Cui *et al.*, degradation of Bronopol in natural waters would be in the range of 0.03 h half-life (k deg 19.51-22.39 h^{-1}, geometric mean = 20.66 h^{-1}):

Photolysis kinetics of bronopol (BNP) and 2-bromo-2-nitroethanol (BNE) in natural waters ($R > 0.989$, $P < 0.0001$).

Water sample	BNP				BNE[a]			
	Degradation	Hydrolysis	Photolysis		Degradation	Hydrolysis	Photolysis	
	K_T (h^{-1})	K_T (h^{-1})	K_T (h^{-1})	$t_{1/2}$ (h)	K_T (h^{-1})	K_T (h^{-1})	K_T (h^{-1})	$t_{1/2}$ (h)
1	19.513	17.952	1.561	0.444	0.052	0.021	0.031	22.360
2	22.393	20.729	1.664	0.416	0.062	0.018	0.044	15.753
3	20.506	17.768	2.738	0.253	0.071	0.022	0.049	14.146
4	21.323	19.321	2.002	0.346	0.122	0.013	0.109	6.359
5	19.684	15.444	4.240	0.163	0.061	0.011	0.050	13.863

[a] Represents degradation rates of BNE after reaching maximum concentrations, based on the suppose that degradation of the compound was ignored before reaching maximum concentrations.

Table A-43: Summary table - Freshwater aerobic biodegradation

Method, Guideline, GLP status, Reliability, Key / supportive study	Test type[1]	Exposure	Test substance concentration	Incubation period	Degradation (DT50)	Remarks	Reference
			Bronopol		0.034 h		Cui *et al.*, Toxicity profile of labile preservative bronopol in water: The role of more persistent and toxic transformation products, Environmental Pollution (2010), doi:10.1016/j.envpol.2010.09.036

[1] *Test according to OECD criteria*

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biodegradation in freshwater, aerobic aquatic degradation study (Annex II, Title 1, Point 10.1.3.2a)

Justification	The existing hydrolysis and ready biodegradation studies with [^{14}C]Bronopol, together with literature data, demonstrate that rapid primary degradation of environmentally realistic concentrations occurs. In conclusion, running an additional study on biodegradation according to the methods mentioned above would provide little additional insight into the environmental fate of Bronopol beyond what is already available.

Water/sediment degradation test

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biodegradation in freshwater, water/sediment degradation test (Annex II, Title 1, Point 10.1.3.2b)
Justification	Substantial mineralization of the compound was observed in a ready biodegradation test (51 to 57% CO_2 was produced in 28 days). Moreover, Bronopol does not adsorb to sediment to any relevant extent, judging from its very moderate K_{OC} of 136 mL/g. Therefore, no water/sediment study is necessary.

A.4.1.1.3.3 Biodegradation in seawater

Seawater degradation study

Applicants presented a data waiving based on old unplubished studies. For classification purposes this is accepted as the behaviour in seawater is similar to natural waters and hence is considered covered. Further, Bronopol is unlikely to reach seawater, from the expected uses of the products.

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biodegradation in sea water (Annex II, Title 1, Point 10.1.3.3)
Justification	A study on the stability of bronopol in sea and estuarine water was undertaken and reported by [redacted] (1984; A7.1.1.1_01). The results of the study indicated that for bronopol in seawater nothing else than the normal degradation for the tested pH range has to be expected; i.e., factors influencing the stability of bronopol in seawater are the same as for freshwater. [redacted] (1996; A7.1.1.1.1_01_a) reported that at pH of 7 or 9, bronopol tested at 10 to 100 ppm had a half-life of 2.4 hours, indicating a rapid initial hydrolysis. Seawater is known to be slightly alkaline (i.e., pH about 8.0 to 8.6; due to the natural buffering from the carbonate and bicarbonate dissolved in the water) and therefore, a rapid initial rate of hydrolysis of bronopol also can be expected in seawater. All this data is indicative of low stability of bronopol in seawater; therefore, any indirect emissions via freshwater to marine water are negligible. Direct emission can be excluded as Bronopol is not used in or released into marine environments. Accordingly, there is no need in conducting a biodegradation study in seawater. References: [redacted] 1984 (A7.1.1.1_01) and [redacted] 1996 (A7.1.1.1.1_01_a)

A.4.1.1.3.4 Higher tier degradation studies in water or sediment

No data available.

A.4.1.1.3.5 Biodegradation during manure storage

Not applicable for the CLH report.

A.4.1.1.3.6 Biotic degradation in soil

A.4.1.1.3.6.1 Laboratory soil degradation studies

Aerobic biodegradation

Although no degradation study into soil was performed for Bronopol, the study from [redacted]

(1992; A7.1.3_01) showed that bronopol does not adsorb effectively to soil and rather is instable in this compartment. Further, bronopol is highly water-soluble and mainly distributed in the compartment water (> 99%), as shown by Mackay Level I calculation ([redacted] 2006; no BPD-ID), where it is susceptible to both hydrolysis and biodegradation; therefore, in soil, bronopol rather has to be expected in the soil pore water, where it would be subject to rapid initial hydrolysis. Besides, inherent biodegradability of Bronopol was shown in the ready biodegradability test where substantial mineralisation of the compound was observed (51 to 57% CO_2 was produced in 28 days). Therefore, stability of Bronopol in soil is unlikely.

Regarding degradation products in soil, in the study of [redacted] (1992; A7.1.3_01), comparing loam and clay loam as the two soils with the highest clay content and therefore the greatest potential adsorptive capacity, higher adsorption was observed in alkaline soil (loam) than in acidic soil (clay loam) due to differences in the degradation pathway of Bronopol. In fact, in the more alkaline soil (sand), relatively non-polar degradation products (such as 2-bromo-2-nitroethanol) were observed which might be potentially more adsorptive than the relatively polar products (such as tris(hydroxymethyl)nitromethane) formed in the more acidic soils (loamy sand and clay loam).

In conclusion, it has been shown that adsorption to soil differs among the various soil types, however, the absorption potential is generally low, with a low coefficient for adsorption (Koc = 136 L/kg).

Anaerobic biodegradation

Data waiving	
Information requirement	Fate and behaviour in soil, laboratory study on rate and route of degradation including identification of the processes involved and identification of any metabolites and degradation products in one soil type (Annex II, Title 1, Point 10.2.1)
Justification	According to the use patterns of Bronopol, exposure to anaerobic conditions is unlikely. No anaerobic biodegradation study needs to be conducted.

A.4.1.1.3.6.2 Higher tier degradation studies in soil

Field dissipation studies (field studies, two soil types)

Data waiving	
Information requirement	Fate and behaviour in soil, field studies, two soil types (Annex II, Title 1, Point 10.2.2)
Justification	Bronopol releases to soil are negligible via direct pathways when it is applied as recommended for the use patterns. In addition, Bronopol is highly water-soluble and mainly distributed in the compartment water ([redacted] 2006; no BPD-ID) where it is susceptible to both hydrolysis and biodegradation. Consequently, no field studies on dissipation in soil are needed. Reference: [redacted] 2006 (no BPD-ID)

A.4.1.1.3.7 Short summary and overall relevance of the provided information on degradation and conclusion on rapid degradation for classification and labelling purposes

Abiotic degradation

The two valid studies for hydrolysis of Bronopol ([redacted] 1996-A7.1.1.1.1_01 and [redacted] 2003-A7_1_1_1_1-01) according to OECD TG 111 at pH 4, 7 and 9 at 50°C, demonstrated a rapid hydrolisation at high pH and T (hydrolysis of Bronopol was both pH and concentration dependent). The hydrolysis rate increased whilst substance concentration decreased. At pH 7, half-life of 0.0245 days was derived by using CAKE 3.4 software at 50°C (r^2 = 0.9474).

At pH 4, the Bronopol molecule was intrinsically more stable, but results showed a steady hydrolysis with time and a similar though less marked concentration effect.

In addition to those standard tests, there are some publications available (Challis and Yousaf, and Cui), which confirms the results obtained in the tests, such as the hydrolysis of Bronopol takes place very rapidly at environmental relevant pH values, with a significant pH and concentration dependency displaying an accelerated rate of hydrolysis at lower concentrations and elevated pH, and provide some possible degradation pathways, most of them implying the formation of a transient product, 2-bromo-2-nitro-ethanol (BNE, whose concentration remained constant in equilibrium with the parent), with a further decomposition to Bromonitromethane (BNM) and 1-bromo-1-nitroethene (detected as secondary peaks). Other degradation pathways lead to the possible formation of Tris(hydroxymethyl)nitromethane (TNM), Glycolic acid, Formic acid, Methanol (all <5%, 24 h) and 2,2-Dinitroethanol (<1%).

The decomposition of BNE occurs at a much slower rate than the parent compound Bronopol.

Formaldehyde is involved in many processes, a number of reactions involving formaldehyde occurs simultaneously. The overall result is that the formaldehyde concentration tends to a maximum which is lower than an equimolar ratio.

Regarding photolysis, with a half-live of 20 days, under natural conditions, hydrolysis and primary biodegradation in water are expected to be more rapid than photolysis of Bronopol. This is especially true taking into account that sunlight penetrates only the very uppermost layers of natural waters. Therefore, potential phototransformation products of Bronopol are expected to occur only at negligible levels in the environment.

Bronopol has a very low Henry´s Law constant of $1.16*10^{-6}$ Pa*m^3/mol at 25°C (calculated with EPI suite 4.1.1) and therefore volatilisation is not to be expected.

The rate constant for phototransformation of Bronopol in air was estimated using the AOPWIN software with a tropospheric half-life of 24.2 days calculated for reaction of OH-radicals with Bronopol, assuming 12 h of sunlight, 25°C, and an OH-radical concentration of $0.5*10^6$ cm^{-3} ([redacted] 2007; A7_3_1-01). Based on this, Bronopol will be rather slowly degraded in air.

Biotic degradation

Bronopol's potential of readily biodegradation was investigated in several studies according to different methods described in the OECD guideline 301:

- Modified OECD 301B test ([redacted] 2002a; A7_1_1_2_1-01): considerable mineralisation of Bronopol of 51-57% was observed within the 28-day test period. Accordingly, the classification target of 60% mineralisation is not met and Bronopol can thus not be classified as readily biodegradable.

- Ready biodegradability OECD TG 301B ([redacted] 1999; A7.1.1.2.1_01): 45-55% mineralisation was achieved within the 10-day-window, and 67-89% after a period of 29 days.

- In 2022, the biodegradation behaviour of Bronopol was again investigated in a GLP study according to a modified procedure based on OECD TG 301B ([redacted] 2022; A7.1.1.2.1_02). After the exposure period of 28 days, 3% degradation was observed in the abiotic control and 20% degradation in the test mixtures with Bronopol missing the threshold for readily biodegradability (60% at the end of 10 day-window) as well as for ultimately biodegradability (60% at test end).

While the criteria for readily biodegradability was not fulfilled in neither of these studies, signs of abiotic degradation (e.g. hydrolysis) were observed indicating that abiotic

degradation processes are predominant under the respective test conditions and thus at environmental relevant pH values. At higher test concentrations inhibition of the microbial inoculum was observed.

Since rapid degradation occurred via both biotic and abiotic pathways it can be concluded that Bronopol will not persist in the environment.

According to CLP guidance, a substance is considered to be not rapidly degradable unless at least one of the following is fulfilled:

a. The substance is demonstrated to be readily biodegradable in a 28-day test for ready biodegradability: this is not the case for Bronopol.

b. The substance is demonstrated to be ultimately degraded in a surface water simulation test with a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days): there is no degradation in surface water test available.

c. The substance is demonstrated to be primarily degraded biotically or abiotically e.g. via hydroysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70 % within 28 days), and it can be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment.

Bronopol meets point c. first part, that is, is rapidly abiotically degraded, but the degradation product BNE is toxic to the environment and fulfil the criteria for classification as hazardous to the aquatic environment. Hence, Bronopol must be considered as not rapidly degradable for classification purposes.

A.4.1.2 Distribution

A.4.1.2.1 Adsorption onto/desorption from soils

Table A-44: Summary table - Adsorption/desorption *

Method, Guideline, GLP status, Reliability, Key/supportive study	Soil	Adsorbed AS [%]#	(K_a) K_{aoc}	(K_d) K_{doc}	K_a/K_d	K_f	1/n	Remarks	Reference
OECD 121 (HPLC method), GLP, Rel. 2	No soil	n.a.	log K_{OC} = 1.0; K_{OC} = 10	n.a.	n.a.	n.a.	n.a.		[redacted] 2002c (A7_1_3-01)
OECD 121 (HPLC method), GLP, Rel. 3	No soil	n.a.	log K_{OC} = 1.07	n.a.	n.a.	n.a.	n.a.		[redacted] 2000 A7_1_3-02)
US EPA OPP 163-1, GLP, Rel. 2 Key	Soil 1: sand, pH 8.2	<8%	(0.2284-0.8329) 388.3-1416	(4.6156-25.160) 7847-42773	0.03-0.05	0.5356	0.4654	**Degradation products** **Name+** / **[%] of a.s.**	[redacted] 1992 (A7.1.3_01)

								Comp. B 54.26 Comp. A (BNE) 24.85 Comp. C (TNM) 9.88 Comp. D 9.42
	Soil 2: loamy sand, pH 4.7	<13%	(0.7149-0.9477) 46.74-61.97	(11.927-16.264) 779.9-1063	0.056-0.067	0.8965	0.8865	**Degradation products** **Name⁺ [%] of a.s.** Comp. B - Comp. A - Comp. C (TNM) 95.57 Comp. D 2.34
	Soil 3: loam, pH 7.0	<26%	(1.9756-3.5455) 170.5-306.0	(20.204-31.131) 1744-2686	0.077-0.114	2.541	0.7863	**Degradation products** **Name⁺ [%] of a.s.** Comp. B 64.11 Comp. A - Comp. C (TNM) 25.46 Comp. D 7.85
	Soil 4: clay loam, pH 5.9	<25%	(1.1879-1.2636) 36.82-41.31	(8.8943-10.696) 290.8-349.7	0.118-0.133	1.198	0.9842	**Degradation products** **Name⁺ [%] of a.s.** Comp. B 5.86 Comp. A - Comp. C (TNM) 84.85 Comp. D 8.55

K_a = Adsorption coefficient
K_{aOC} = Adsorption coefficient based on organic carbon content
K_d = Desorption coefficient
K_{dOC} = Desorption coefficient based on organic carbon content
K_a/ K_d = Adsorption / Desorption distribution coefficient
n.a. = not applicable for the HPLC method

[#] (Mean of 4 test substance conc.; 2 samples each): sum of supernatant desorption; soil extract and soil residue given as % radioactivity of initial concentration

[+] Component A was tentatively identified as 2-bromo-2-nitroethanol (BNE); Component C was chromatographically similar to tris(hydroxymethyl)-nitromethane (TNM)

Adsorption of ^{14}C-Bronopol onto and desorption from four different soil types was investigated ([redacted], 1992; A7.1.3_01), according to US EPA Guidelines, Subdivision N,

Paragraph OPP 163-1. The results indicated that highest adsorption was observed in loam. Lower adsorption was observed in clay loam, loamy sand and sand. From the $K_a$oc values of the four different soil types, a Koc of 136.06 L/kg (geometric mean) was calculated for Bronopol. According to the classification developed by FAO[3] (2000) and recommended by US-EPA[4] for the mobility in soil, Bronopol is considered as moderately mobile.

Moreover, in the study of [redacted] (1992; A7.1.3_01) the test material was instable under test conditions and the observed degradation varied in the four soil types. Adsorption of Bronopol and/or its degradation products was correlated to the soil pH. Comparing loam and clay loam as the two soils with the highest clay content and therefore the greatest potential adsorptive capacity, higher adsorption was observed in alkaline soil (loam) than in acidic soil (clay loam) due to differences in the degradation pathway of Bronopol. In fact, in the more alkaline soil (sand), relatively non-polar degradation products (such as 2-bromo-2-nitroethanol) were observed which might be potentially more adsorptive than the relatively polar products (such as tris(hydroxymethyl)nitromethane) formed in the more acidic soils (loamy sand and clay loam).

In the two supporting studies ([redacted] 2002c-A7_1_3-01 and [redacted] 2000-A7_1_3-02), the log Koc of Bronopol was estimated by comparison of capacity factors determined on HPLC with a CN-column for seven reference compounds with known Koc values to the capacity factor of Bronopol on the same HPLC system. The procedure was in line with the OECD TG 121. Additionally, the log Koc of Bronopol was also estimated with two commercial QSAR softwares using first-order molecular connectivity indexes. Very low log Koc values of 1 and 1.07 for Bronopol were determined in the HPLC-method according to OECD TG 121 resulting in Koc values of 10 and 11.75. But as the determined log Koc values lay below the range of the log Koc values for the reference compounds (lowest values for log Koc: 1.45 for 2-Nitrobenzamide and 1.25 for acetanilide), these values can only be considered as first estimates. The results of the QSAR calculations for the log Koc varied and lay in the range of 0-2.3 (corresponding Koc values 1-199) corroborating the results from the HPLC-method as well as from the experimental study by [redacted] (1992; A7.1.3_01).

In conclusion, it has been shown that adsorption to soil differs among the various soil types, however, the absorption potential is generally low, indicating no obvious risk to the soil compartment.

Adsorption onto / desorption from soils – metabolites

No studies are available on the adsorption in soil and sediments for neither of the main metabolites (BNE, TNM, formaldehyde).

For BNE and TNM, low K_{OC} values of 2.826 and 10 L/kg were estimated by MCI in KOCWIN. For formaldehyde, the K_{OC} was estimated with the QSAR model described in EU Technical Guidance Document on Risk Assessment (EC 2003). Based on a log P_{OW} of 0.35 and the QSAR for non-hydrophobics, the K_{OC} is calculated to be 15.9 L/kg.

Additionally, a calculation of the fugacity model (Level 1) according to Mackay (1991), using the “unit world” given in Jørgensen & Bendoricchio (2001), demonstrates that formaldehyde is preferentially distributed to the hydrosphere (98.7%). Distribution into other environmental compartments is of secondary importance.

In conclusion, the available data on adsorption/desorption is sufficient for the risk assessment. Adsorption of relevant amounts of BNE, TNM and formaldehyde on soils and

[3] FAO (2000) Appendix 2. Parameters of pesticides that influence processes in the soil. In FAO Information Division Editorial Group (Ed.), Pesticide Disposal Series 8. Assessing Soil Contamination. A Reference Manual. Rome: Food & Agriculture Organization of the United Nations (FAO). Accessed August 5, 2022.
[4] USEPA (2006) Standard Soil Mobility Classification Guidance. Memorandum From S. Bradbury to Environmental Fate and Effects Division. January 23, 2004. Environmental Fate and Effects Division. Office of Pesticide Programs. United States Environmental Protection Agency.

sediments is not expected.

A.4.1.2.2 Higher tier soil adsorption studies

No data available.

A.4.1.2.3 Volatilisation

Regarding volatilisation, please see Part A, section 1.3 Physical and chemical properties of the active substance.

Due to Bronopol properties, it is not expected to volatilise. It was shown by a Mackay Level I calculation that Bronopol is mainly distributed in the compartment water (>99%). Therefore, the atmosphere is considered to be no relevant compartment for the occurrence of Bronopol. For those low quantities of Bronopol reaching the atmospheric compartment, the substance will be slowly photodegraded in air in photochemical processes, with a tropospheric half-life of Bronopol calculated by using the AOPWIN program (Version 1.91) of 12 days.

A.4.1.3 Bioaccumulation

The n-octanol/water partition coefficient (Pow) of Bronopol is 0.48, 0.38 and 0.31 (log Pow -0.32, -0.42, -0.50) at 10, 20 and 30°C, respectively. Therefore, the substance is considered to have a negligible potential for bioconcentration due to its lack of lipophilicity. In general, for substances with a log Pow < 3 the experimental determination of the BCF is not required. Furthermore, Bronopol is not surface active (surface tension of 72 mN/m at a concentration of 1.0 g/L).

This assumption is also supported by the high water solubility of Bronopol, i.e., 304 g/L at 20°C, limiting the substance's affinity to partition to lipid compartments and thus the probability of bioconcentration.

The estimation of the BCF via models based on the log Pow is only recommended for substances with a log Pow of 1-10 (ECHA Guidance on information requirements, Chapter R.7c, Version 3.0). Therefore, the estimation method is not applicable for Bronopol.

Further, QSAR modelling gives a very low Log Octanol-Water Partition coefficient (SRC): Log Kow (KOWWIN v1.67 estimate) = -0.64

For the main transient product, BNE, QSAR modelling gives as well a very low Log Kow (KOWWIN v1.67 estimate) = -0.74 and for the main metabolite, TNM, Log Kow (KOWWIN v1.68 estimate) = -1.66.

Hence, no bioaccumulation is expected.

In case of soil organisms, Bronopol is expected to remain in the pore water based on the high water solubility and the low log Pow. Hence, measuring terrestrial bioconcentration is therefore not relevant.

Measured aquatic bioconcentration

No data available.

A.4.1.3.1 Short summary and overall relevance of the provided information on bioaccumulation and conclusion on bioaccumulation potential for classification and labelling purposes

No bioaccumulation studies were submitted as no bioaccumulation is expected due to the high water solubility and vey low LogKow for Bronopol and its degradation products (for the main transient product, BNE, QSAR modelling provides Log Kow (KOWWIN v1.67 estimate) = -0.74 and for the main metabolite, TNM, Log Kow (KOWWIN v1.68 estimate) = -1.66).

A.4.1.4 Monitoring data

Monitoring data sewage, surface water and air

Monitoring data for Bronopol in the environment in Sweden were published by Remberger (2006). Overall, samples from 58 sites and 11 matrices were analysed for Bronopol, and the active substance was always below the limit of detection (LOD) of the respective matrix. Most samples (42 of 58) comprised matrices exposed to diffuse or point source emissions from anthropogenic activities. The results pertinent to the present dossier are summarised in the table below.

Matrix analysed	General pollution level	No. of sites/ samples of different origin analysed	Findings
Sewage treatment plant effluents	Diffuse and point source emissions	5	< LOD of 0.05 µg/L
Surface water	Natural background	3	< LOQ of 0.05 µg/L
	Diffuse and point source emissions	1	
Sewage sludge	Diffuse and point source emissions	8	< LOQ of 12-24 µg/kg dry wt
Sewage water	Diffuse and point source emissions	1	< LOD of 0.05 µg/L
Air	Natural background	6	< LOD of 0.05-0.07 ng/m^3
	Diffuse and point source emissions	3	
Precipitation	Natural background	2	< LOD of 0.16 µg/L
	Diffuse and point source emissions	1	

Due to the widespread use of Bronopol, it is to be expected that Bronopol was also used in the regions in Sweden where sampling was carried out. Therefore, if Bronopol would be persistent in the environment, findings above the LOD would have to be expected. This was the case for other biocidal active substances analysed in the monitoring study.

However, no Bronopol could be detected in a large variety of environmental matrices from Sweden. This indicates that the existing uses of Bronopol lead to negligible environmental concentrations below the relevant PNEC value in water (0.5 µg/L).

A.4.2 Effects on environmental organisms

A.4.2.1 Atmosphere

Not applicable for the CLH report.

A.4.2.2 Toxicity to sewage treatment plant (STP) microorganisms

Inhibition of microbial activity (aquatic)

Table A-45: Summary table - Inhibition of microbial activity

Method, Guideline, GLP status, Reliability, Key/supportive study	Species/ Inoculum	Endpoint	Exposure		Results			Remarks	Reference
			Design	Duration	NOEC	EC10	EC50		
88/302/EEC, GLP, Rel. 2	activated sludge from domestic sewage treatment plant	Respiration inhibition	static	3 hours	Not stated	4 mg a.s./L	11 mg a.s./L	GLP, dose response, nominal conc.	[redacted] 2000b (A7_4_1_4-01)
OECD 209 (1993), GLP, Rel. 2	Activated sludge	Respiration inhibition	static	30 min	Not stated	Not stated	About 230 mg/L	Nominal Conc.	[redacted] 2002 (A7.4.1.4_01)
OECD 209 (1984), GLP, Rel. 1 Key	Activated sludge	Respiration inhibition	static	2.5 hours	Not stated	Not stated	43 mg/L	Nominal Conc.	[redacted] 1996 (A7.4.1.4_02)
ISO 10712, non-GLP, Rel. 2	*Pseudomonas putida*	Growth inhibition	static	16±1 hours	Not stated	0.5 mg/L	2.33 mg/L	Nominal Conc.	[redacted] 1996 (A7.4.1.4_03)

The effect of Bronopol on aquatic microorganisms (activated sludge) was investigated in a study according to OECD TG 209 ([redacted] 1996; A7.4.1.4_02). After test duration of 2.5 hours at test concentrations ranging from 2 to 200 mg/L, the EC_{50} value was nominal 43 mg/L. The validity criteria of the test can be considered as fulfilled, since the EC_{50} for the reference substance 3,5-dichlorophenol was estimated to be 12 mg/L (required in the range 5 to 30 mg/L) and the two blank controls had respiration rates within 15% of each other. But at intermediate concentrations the validity criterion is bigger than 15%. The parameter temperature and pH are slightly above the recommended values in the guideline 209. This study was considered as key study, because of its better quality and smaller deficiencies (it included a preliminary study, comply with the two control replicates, better performance quality).

The community of aquatic microbial destruents reacted moderately sensitive to Bronopol as indicated by a 50% decrease in the respiration activity at a concentration of 11 mg a.s./L ([redacted] 2000b; A7_4_1_4-01). The test was performed with a guidance equivalent to OECD 209. The test is considered acceptable despite certain deficiencies were found.

In a further OECD 209 study ([redacted] 2002; A7.4.1.4_01), toxicity to aquatic microorganism (activated sludge) toxicity was assessed. At nominal test concentrations ranging from 10 to 1000 mg/L, inhibition of respiration in activated sludge ranged from 14 to 67%. After a test duration of 30 min, the EC_{50} value was 230 mg/L. The validity criteria of the test system were fulfilled, since deviations of blank controls were less than 15%, and the EC_{50} of the reference substance 3,5-dichlorophenol was within the range of 5 to 30 mg/L according to the OECD guideline. Some small deficiencies were found in the test (no measured concentrations, only nominals and just one replicate for each concentration, and no preliminary study was performed).In a third study, the effect of Bronopol on the growth of *Pseudomonas putida* was studied according to ISO guideline 10712 ([redacted] 1996; A7.4.1.4_03). The concentrations tested ranged from 0.39 to 12.5 mg/L. After 16 hours of incubation, the EC_{50} was nominal 2.33 mg/L. Due to a sufficient performance of the reference substance (3,5-dichlorophenol) with an EC_{50} of 28.7 mg/L (10 to 30 mg/L required) and an increase of the control culture by 59.7 times during the test (increase of 60 times required), the test can be regarded as valid. This study is not GLP. According to the guidance, this study is only to be used if no other study is available, which is not the case.

A.4.2.4 Aquatic compartment

A.4.2.4.1 Freshwater compartment

Acute / short-term toxicity (freshwater)

Table A-46: Summary table - acute/short-term aquatic toxicity

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
Fish								
OECD 203 (1992), US EPA OPPTS 850.1075 (1996), GLP, Rel. 2 Key	Bluegill sunfish, *Lepomis macrochirus*	Mortality / acute	Bronopol	flow-through	96 hours	LC_{50} = 11 mg/L	dose-response, mean measured conc.	[redacted] 2006a (A7_4_1_1-01)
US EPA OPP 72-1, GLP, Rel. 2	*Lepomis macrochirus*	Mortality	Bronopol	flow-through	96 hours	LC_{50} = 35.7 mg/L	Measured conc.	[redacted] 1984 (A7.4.1.1_02)
OECD 203 (1992), US EPA (1996) OPPTS 850.1075, GLP, Rel. 2	Rainbow trout, *Oncorhynchus mykiss*	Mortality / acute	Bronopol	flow-through	96 hours	LC_{50} = 26.4 mg/L	dose-response, mean measured conc.	[redacted] 2005 (A7_4_1_1-02)
US EPA OPP 72-1, GLP, Rel. 2	*Oncorhynchus mykiss* (formerly *Salmo gairdneri*)	Mortality	Bronopol	flow-through	96 hours	LC_{50} = 41.2 mg/L	Measured conc.	[redacted] 1984 (A7.4.1.1_01)
Method comparable to OECD 203, non-GLP, Rel. 2	*Oncorhynchus mykiss*	Mortality	Tris-hydroxymethyl-nitromethane (Tris Nitro, TNM)	static	96 hours	LC_{50} = 410 mg/L	Nominal conc.; Performance of study before implementation of GLP and adoption of OECD guideline	[redacted] 1973 (A7_4_1_1-03)
OECD 203 (1992), GLP, Rel. 2	*Oncorhynchus mykiss*	Mortality	2-bromo-2-nitroethanol (BNE)	Flow-through	96 hours	LC_{50} = 3.0 mg/L	Mean measured conc.	[redacted] 2012 (A7_4_1_1-04)
US EPA OPP 72-1, GLP, Rel. 2	Sheepshead minnow *Cyprinodon variegatus*	Mortality	Bronopol	flow-through	96 hours	LC_{50} = 57.6 mg/L	Measured conc.	[redacted] 1984 (A7.4.1.1_03)

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC / EC_{50}		
Invertebrates								
92/69/EEC C.2 (1992), GLP, Rel. 2 Key	Water flea, *Daphnia magna*	Immobility / acute	Bronopol	static	48 hours	EC_{50} = 1.04 mg/L (geomean), 1.32 mg/L (initial measured)	dose-response, measured conc.	[redacted] 2000 (A7_4_1_2-01)
Method comparable to OECD 202, GLP, Rel. 2	*Daphnia magna*	Immobility	Bronopol	static	48 hours	EC_{50} = 1.4 mg/L	Nominal conc.	[redacted] 1981 (A7.4.1.2_01)
US EPA 72-2, GLP, Rel. 2	*Daphnia magna*	Immobility	Tris-hydroxymethyl-nitromethane (Tris Nitro, TNM)	static	48 hours	EC_{50} = 80 mg/L	Nominal conc.	[redacted] 1989 (A7_4_1_2-03)
OECD 202 (2004), GLP, Rel. 2	*Daphnia magna*	Immobility	2-bromo-2-nitroethanol (BNE)	Flow-through	48 hours	EC_{50} = 0.38 mg/L	Mean measured conc.	[redacted] 2012 (A7_4_1_2-04)
OPPTS 850.1035 (1996), GLP, Rel. 1	Marine crustacea, *Americamysis bahia*	Immobility / acute	Bronopol	flow-through	96 hours	48h-EC_{50} = 7.9 mg/L (mean measured); 96h-EC_{50} = 4.3 mg/L (mean measured)	dose-response, measured conc.	[redacted] 2006 (A7_4_1_2-02)
ISO 14669, GLP, Rel. 3	Marine copepod, *Acartia tonsa*	Immobility	Bronopol	static	48 hours	EC_{50} = 3.5 mg/L	Nominal conc.	[redacted] 1998 (A7.4.1.2_02)
Algae (growth inhibition)[1]								
OECD 201 (1984), 92/69/EEC Method C.3 (1992), US EPA OPPTS 850.5400 (1996), GLP, Rel. 2 Key	Freshwater green microalga, *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.0073 mg/L (geomean)	measured concentration	[redacted] 2006a (A7_4_1_3-01)
OECD 201 (1984), GLP, Rel. 3	*Desmodesmus subspicatus* (formerly	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} > 1.0 mg/L	Nominal conc., no analytical monitoring	[redacted] 1994 (A7.4.1.3_01c)

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
	Scenedesmus subspicatus)							
OECD 201 (1984), US EPA OPPTS 850.5400, GLP, Rel. 2	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	growth inhibition, Algistatic effects	Bronopol	static	96 hours plus 7 days recovery period	72h-E_rC_{50} = 0.5 mg/L (geomean)	measured conc.	[redacted] 1998 (A7.4.1.3_02)
OECD 201 (1984), 92/69/EEC Method C.3, US EPA OPPTS 850.5400, Rel. 3 GLP	Freshwater bluegreen alga (cyanobacteria), *Anabaena flos-aquae*	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.019 mg/L	geometric mean measured concentration	[redacted] 2006b (A7_4_1_3-02)
OECD 201 (1984), US EPA OPPTS 850.5400, GLP, Rel. 2	Freshwater green microalga, *Raphidocelis subcapitata* (*Pseudokirchneriella subcapitata,* formerly *Selenastrum capricornutum*)	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.035 mg/L (geometric mean measured), 0.233 mg/L (initial measured)	measured concentration	[redacted] 2002 (A_4_1_3-03)
OECD 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (*Pseudokirchneriella subcapitata,* formerly*Selenastrum capricornutum*)	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.37 mg/L	Nominal conc., no analytical monitoring	[redacted] 1994 (A7.4.1.3_01a)
OECD 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (*Pseudokirchneriella subcapitata,* formerly*Selenastrum capricornutum*)	Growth inhibition	2-bromo-2-nitroethanol (BNE)	static	72 hours	72h-E_rC_{50} = 0.109 mg/L (TWA)	Time-weighted average, mean measured conc.	[redacted] 2012 (A7_4_1_3-08)
OECD 201 (1984), Directive 92/69/EEC Method	*Raphidocelis subcapitata* (*Pseudokirchneriella*	Growth inhibition	Tris-hydroxymethyl-nitromethane (Tris	static	96 hours	72h-E_rC_{50} > 4.5 mg/L	Mean measured conc.	[redacted] 2002 (A7_4_1_3-06)

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC / EC_{50}		
C.3, GLP, Rel. 2	*subcapitata,* formerly*Selenastrum capricornutum*)		Nitro, TNM)					
OECD 201 (1984), 92/69/EEC Method C.3, US EPA OPPTS 850.5400, GLP, Rel. 2	Freshwater diatom, *Navicula pelliculosa*	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.135 mg/L	geometric mean measured concentration	[redacted] 2006c (A7_4_1_3-04)
OECD 201 (1984), GLP, Rel. 3	Freshwater green microalga, *Chlorella vulgaris*	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.89 mg/L (first test), 2.84 mg/L (second test)	Nominal conc. due to inconsistencies in analytical measurement	[redacted] 1994 (A7.4.1.3_01b)
OECD 201 (1984), US EPA OPPTS 850.5400, GLP, Rel. 2	Marine diatom, *Skeletonema costatum*	growth inhibition	Bronopol	static	96 hours	72h-E_rC_{50} = 0.052 mg/L	geometric mean measured concentration	[redacted] 2006d (A7_4_1_3-05)
ISO 10253, US EPA OPPTS 850.5400, GLP, Rel. 2	*Skeletonema costatum*	growth inhibition Algistatic effects	Bronopol	static	72 hours plus 7 days recovery period	72h-E_rC_{50} = 0.25 mg/L	Nominal conc., no analytical monitoring	[redacted] 1998 (A7.4.1.3_03)
Other aquatic plants								
OECD 221 (proposal April 2004), US EPA OPPTS 850.4400, GLP, Rel. 2	Freshwater duckweed, *Lemna gibba*	frond growth	Bronopol	static	7 days	E_rC_{50} = 142 mg a.s./L	Dose-response	[redacted] 2006e (A7_4_3_5_2-01)

Description of the available acute toxicity studies

Acute (short-term) toxicity to fish

Acute effects of Bronopol to fish were investigated in a warm- and a cold-freshwater species as well as in one saltwater species.

The acute toxicity of Bronopol to rainbow trout (cold-freshwater, *Oncorhynchus mykiss*, formerly *Salmo gairdneri*) was tested in two flow-through tests at nominal 10, 18, 32, 56, 75, 100 and 180 mg/L (1984; A7.4.1.1_01) and 4.28, 7.13, 11.9, 19.8, 33.0 and 55.0 mg/L (2005; A7_4_1_1-02) according to US EPA Guideline OPP 72-1 and OECD 203, respectively. While in the study of (1984; A7.4.1.1_01) the LC50 was 41.2 mg/L and the NOEC 23 mg/L (both based on measured concentrations), in the study of (2005; A7_4_1_1-02) all fish in the highest concentration and 50% of the fish in the next two lower treatments died and adverse effects were observed down to nominal 11.9 mg/L, resulting in a NOEC of 7.82 mg/L (measured) and a LC50 of 26.4 mg/L.

The acute toxicity of Bronopol to bluegill sunfish (warm-freshwater, *Lepomis macrochirus*) was tested in two flow-through tests at nominal 7.5, 10, 18, 32, 56 and 75 mg/L (1984; A7.4.1.1_02) and 5.5, 10, 18, 33 and 60 mg/L (2006a; A7_4_1_1-01) according to US EPA Guideline OPP 72-1 and OECD 203, respectively. In the study by (1984; A7.4.1.1_02), symptoms of toxicity occurred at a concentration of 20 mg/L (measured), resulting in a NOEC of 11.4 mg/L (measured). In the study by (2006a; A7_4_1_1-01), all fish in the highest concentration and 85% of the fish in the next lower treatment died. As in the concentration of nominal 18 mg/L still 5% of the fish died, the NOEC was determined as 2.9 mg/L (measured). This is selected as the key fish study, with a LC_{50} = 11 mg/L.

The acute toxicity of Bronopol was also studied in the marine fish sheepshead minnow (*Cyprinodon variegatus*). Again, the test was carried out according to US EPA Guideline OPP 72-1 in a flow-through test at nominal concentrations of 18, 32, 56, 75, 100 and 180 mg/L (1984; A7.4.1.1_03). Symptoms of toxicity were observed at a concentration of 16.9 mg/L (measured), resulting in a NOEC of 8.5 mg/L (measured) and the LC50 was determined as 57.6 mg/L.

Bronopol displayed a low toxicity towards both fresh- and saltwater fish species with LC_{50} values ranging from 11 to 57.6 mg/L. The NOEC is given for informative purpose as this parameter is of lower relevance for short-term studies. The results of the studies according to OECD TG 203 are lower than the ones from the studies according to US EPA OPP 72-1, which is no longer valid but was replaced by the US EPA OPPTS 850.1075. Assuming that methodology has improved over time, the results from the “younger” OECD TG 203 are considered more reliable. Between the two different freshwater species – bluegill sunfish and rainbow trout - no significant difference in sensitivity concerning short-term effects was determined.

Regarding metabolites, a study on rainbow trout was performed with Tris(hydroxymethyl)nitromethane (, 1973; A7_4_1_1-03), with no GLPs or analytical confirmation of the concentration tested. The materials and methods are vaguely described in the report. Mortality in the controls was <10% and dissolved oxygen was greater than 60%. No analytical confirmation of exposure concentrations was conducted. As TNM is relatively stable in freshwater (DT50 hydrolysis at 25 ºC and pH 7 = 3.4 days, and 11.3 d at 12ºC), this lack of analytical data is not invalidating the study, but the results should be considered carefully. The test clearly shows a low effect of Tris(hydroxymethyl)nitromethane to fish. Additionally, two public studies have been consulted by the applicants as supporting information (A7.4.1.1_04). The EPA considered the studies as “Valid Without restriction” and they gave a NOEC of 180 and 501 mg/L showing a low effect of Tris(hydroxymethyl)nitromethane to fish.

A study on acute toxicity of 2BNE to *Oncorhynchus mykiss* following the OECD 203 guideline

was performed ([redacted] 2012; A7_4_1_1-04). Following 48 hours of exposure, 100% mortality was observed among fish exposed to the 4.3 mg/L treatment level. At test termination, no mortality or adverse effects were observed among fish exposed to any of the remaining treatment levels (0.26, 0.52, 1.2 and 2.1 mg/L) or the control. Based on mean measured concentrations, the 96-hour LC50 value for rainbow trout (*Oncorhynchus mykiss*) to 2-bromo-2-nitroethanol was determined by binomial probability to be 3.0 mg/L (95% CI 2.4 - 3.8 mg/L). The NOEC was determined to be 2.1 mg/L. Nevertheless, the curve fit is not good because a concentration around the expected LC50 has not been tested. The LC100 is considered to be 4.3 mg/L but it could be lower since at 24 h all fish are dead or lethargic, and by 48 hours all are dead. The NOEC is not enough supported because the clinical signs have not been reported as established TG OCDE 203, and the observations were fewer than requested in the TG. Therefore, the endpoints should be considered carefully.

Acute (short-term) toxicity to aquatic invertebrates

Acute effects of Bronopol to aquatic invertebrates were investigated both in freshwater and marine species. The EC_{50} values of freshwater water flea *Daphnia magna* are a little bit lower than those for the marine species which could be an indication for a higher sensitivity of the crustacean species from freshwaters. However, they should be considered with caution due to the steep increase of immobility ([redacted] 1981; A7.4.1.2_01) between two consecutive treatments and the calculation method used, i.e., EC_{50} as geometric mean of EC_0 and EC_{100} ([redacted] 2000; A7_4_1_2-01).

The acute toxicity of Bronopol to *Daphnia magna* was carried out in two static tests with 48 hours exposure period. The study of [redacted] (2000; A7_4_1_2-01) was conducted according to Method C.2 of Directive 92/69/EEC using the nominal test concentrations of 0.5, 1.0, 2.0 and 4.0 mg/L. Actual concentrations at 0 h were <0.022, 0.77, 1.22, 2.25 and 4.55 mg/L, whereas at 48 h concentrations were <0.022, 0.38, 0.76, 1.79 and 4.29 mg/L. As there were only two measurements (start and end of test) and the test substance concentration decreased significantly during the test period, geometric mean concentrations are considered to better reflect the exposure under the test conditions (they have been re-calculated as <0.022, 0.54, 0.96, 2.01 and 4.42 mg/L). The initial measured and the geomean includes parent and metabolites. At the highest test concentration all daphnids were immobilised already after 24 hours of exposure, while at the next lower treatment the full exposure period of 48 hours was necessary to immobilise all daphnids. At the next test concentration of 1.0 mg/L, 55% of daphnids were immobilised after 24 hours increasing to 90% after 48 hours. In the lowest test concentration and the control no daphnid was immobilised. Some deficiencies were found, such as only 2 replicates with 10 daphnia for each concentration has been used; the toxicity values were based on geometric mean (OECD TG p. 26 has been applied, geomean of EC0 and EC100 to obtain EC50) but there is no statistical method to obtain an EC50 with 95% confidence limit. Due to the low number of replicates plus the lack of statistical method, the results should be considered carefully.

The study of [redacted] (1981; A7.4.1.2_01) was conducted by using methods that are comparable to OECD TG 202 and the nominal test concentrations of 0.1, 0.18, 0.32, 0.56, 1.0, 3.2 and 5.6 mg/L. At the highest test concentration all daphnids were immobilised already after 24 hours of exposure, while at the next lower treatment the full exposure period of 48 hours was necessary to immobilise all daphnids. At the next test concentration of 1.0 mg/L, only 5% of daphnids (i.e., one individual) was immobilised after 48 hours and further no daphnids were immobilised in the remaining treatments.4 replicates per concentration were used. The 48 h EC50 value was 1.4 mg/L (nominal), calculated by probit analysis. As no analytical measurements are provided, and between 1 and 3.2 mg/L the effects increase from 5 to 100% immobilisation, the EC50 should be considered carefully as it is based on just two values. It is considered reliable based on the initial measured concentrations in other study with same species and the good performance on replicates and tested concentrations.

The study of [redacted] (1998; A7.4.1.2_02) on the marine copepod *Acartia tonsa* was carried

out at nominal 0.5, 0.8, 1.4, 2.4, 4.0 and 7.0 mg/L Bronopol under static conditions according to ISO guideline 14669, No significant immobility was observed in the controls. Additionally, no significant immobility was detected in the three lowest concentrations after 48 hours before it increased over the three higher concentrations steadily ending up in 100% immobility in the highest treatment. With this clear dose-response-relation, the EC_{50} value could be calculated with a computer program using three different methods (probit analysis, moving average and trimmed Spearman-Karber). Nevertheless, no concentration was determined analytically, and no comparison is possible with other tests with same species. Hence, the study is considered as supporting information.

The second marine species *Americamysis bahia* was tested under flow-through conditions and prolonged exposure of 96 hours to the nominal concentrations of 2.5, 5.0, 10, 20 and 40 mg/L ([redacted] 2006; A7_4_1_2-02). For *Americamysis bahia* a comparable relation could be observed after 48 hours of exposure with 10% immobility in the median concentration, 65% in the second highest and 100% immobility in the highest treatment.

Bronopol showed a moderate acute toxicity towards both fresh- and saltwater invertebrate species with 48h-EC_{50} values ranging from 1.04 to 7.9 mg/L. The results for freshwater species are more relevant for the risk assessment of Bronopol and from the two available studies the study of [redacted] (2000; A7_4_1_2-01) as guideline study with well characterised test material was preferred to the one by [redacted] (1981; A7.4.1.2_01).

Regarding metabolites, a study on acute toxicity to *Daphnia magna* following the OECD 202 guideline was performed with 2BNE ([redacted] 2012; A7_4_1_2-04). Following 48 hours of exposure, immobilization of 10, 100 and 100% were observed in the 0.34, 0.71 and 1.4 mg/L treatment levels (mean measured concentrations), respectively. No immobilization was observed among daphnids exposed to the control or the 0.069 and 0.20 mg/L treatment levels at test termination. No adverse effects were observed among daphnids exposed to the control or the 0.20 mg/L treatment level. Based on mean measured concentrations, the 48-hour EC50 value for Daphnia magna exposed to 2-bromo-2-nitroethanol was determined to be 0.38 mg/L (0.42-0.50 mg/L 95% CI) as a geomean of EC0 and EC100. The NOEC was determined to be 0.20 mg/L. In the flow-through test system, mean measured concentrations ranged from 74 to 100% of targeted nominal concentrations. Nevertheless, the EC50 has been empirically estimated; therefore, the corresponding 95% confidence intervals could not be calculated. Due to the curve fit, the values should be considered carefully.

The test with Tris(hydroxymethyl)nitromethane ([redacted], 1989; A7_4_1_2-03) was performed with six treatment concentrations in duplicate of the test compound (logarithmic series 10 - 180 mg/l, included a control), with ten daphnids (first instar less than 24 hours old) per beaker. All concentrations were observed once every 24 hours for immobility and other abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of test chambers. The test showed low toxicity of this metabolite to invertebrates, with a EC_{50} = 80 mg/L based on nominal concentrations.

Acute (short-term) toxicity to algae or other aquatic plants

Bronopol showed a high acute toxicity towards both fresh- and saltwater algae with E_rC_{50} values ranging from 0.026 to 0.459 mg/L (initial measured), being the lowest value considered 0.0073 mg/L as geometric mean measured concentration, indicating that algae were the most sensitive species within the group of aquatic organisms.

There are several studies available with different species. Due to the rapid hydrolysation of the parent, the geometric mean measured concentrations, when available, are selected. Most of the studies did not include analytical measurements. Taking into account the rapid hydrolysis, the studies are considered reliable when measured concentrations are available for the species tested (in the test considered or in another test with same species). If not, the study would generally be regarded as supporting information.

The key study with *Desmodesmus subspicatus* ([redacted] 2006a; A7_4_1_3-01) provides reliable results with a good statistical analysis. The concentrations used for the EC50 calculations were concentrations measured at time 0 and 72 h. According to section OECD guideline 23, for static and semi-static tests, where the concentrations do not remain within 80-120% of nominal, the effect concentrations could be determined and expressed relative to the geometric mean of the measured concentrations. Also BPR guidance vol IV part B+C indicates in section "3.10 Effects assessment for rapidly degrading substances" that "If measured concentrations at test start and end are available for all concentration levels tested or for the concentration levels that are close to the derived effect value, the geometric mean of the concentrations measured at test start and test end for each treatment may be calculated as an approximation of the actual exposure." Equation 112 or geomean (if only concentrations at test start and end are available, this equation is mathematically equal to the calculation of the geometric mean) should be used. Hence, the geometric mean concentration is more realistic and represents the worst case. The statistically derived EC10 for the key algae study is 0.0048 mg/L (c.i. 0.0041 – 0.0054). The 72h-ErC50 = 0.0073 (ci: 0.0069-0.0076) as geomean and the EC10 = 0.0048 based on geomean (c.i. 0.0041-0.0054) are considered reliable. In this key test, the validity criteria are met (the mean increasing factor in the biomass in the control cultures is higher than 16; the mean coefficient of variation for section-by-section specific growth rates in the control cultures does not exceed 35% except for an individual replicate in section 0-24 h probably due to a lag phase, which can be minimised and practically eliminated in control cultures by proper propagation of the pre-culture; the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures does not exceed 7%). The second validity criterion is not met in one of the sections, which is not considered to affect the test results as the cell density is growing exponentially and the results and the dose-response curves show the effects of the test substance occurring during the exposure period. Further, the statistical analysis of the data performed by this eCA has shown very good results: a good fitting to model log-logistic 4 parameters (and to Weibul 1.4) and good 95% confidence intervals.

Three growth inhibition tests on algae were carried out according to the OECD TG 201 ([redacted] 1994; A7.4.1.3_01a to c). The 72h-E_rC_{50} was 0.37 mg/L for *Raphidocelis subcapitata* (also *Pseudokirchneriella subcapitata* formerly *Selenastrum capricornutum*), 0.89 and 2.84 mg/L for *Chlorella vulgaris* (two test series) and >1.0 mg/L for *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*). All results refer to nominal concentrations since no clear quantification of the actual exposure concentrations could be achieved. The inconsistent results obtained by analytical monitoring may be related to the fact that Bronopol rapidly hydrolyses at pH values ranging from 7 to 9, as shown in other studies (see chapter A.4.1.1.1 Abiotic degradation). In the test c, with *Scenedesmus*, no measured concentrations are provided, and for the same species there are initial measured concentrations 76-103 % from nominals in the test from [redacted] 2006a (A7_4_1_3-01) and 95 - 125 % in the test from [redacted] 1998 (A7.4.1.3_02), but there is an important uncertainty related to the statistical analysis, which indicates that no reliable EC10 or EC50 can be obtained (very wide confidence intervals including value zero for EC10, as well as lack of fit for the lowest part of the curve); hence the test is considered as supporting information. Regarding the test with *Chlorella* (test b), the measured concentrations far differ from nominals and are not detectable at 0.32 mg/L sample at 0 h, and there is no additional test with the same species for a supportive comparison, hence the NOEC is not reliable, and the test is considered as supporting information. The test with *Subcapitata* (test a) is considered reliable as in other test with same species at time 0 concentrations were similar to the nominal (88-98% of the nominals) so the endpoint as nominal here can be accepted and there are no further inconsistencies.

[redacted] 2002 (A_4_1_3-03) performed a test with *Selenastrum capricornutum,* with initial measured concentrations of 0.0151, 0.0323, 0.0615, 0.115, 0.235, 0.465, and 0.939 mg/L. At both the 72- and 96-h intervals, all test solutions were below the MQL (minimum quantifiable limit) of 0.0107 mg/L with the exception of the high dose which had 0.0211 and 0.0165 mg/L, respectively. Algal density at the start of the tests was 10,000 cells/mL.

At 72-hours, Bronopol had no significant effect on algal growth in the two lowest test concentrations (0.017 and 0.033 mg/L nominal) for all three endpoints (cell density, area under the growth curve, and growth rate). There was clear dose-response through the five highest treatment concentrations with nearly complete inhibition at the highest test concentration. A strong dose-response was still seen over the highest remaining test levels. The analytical measurements showed that the concentrations did not maintain within 80% of the nominals. The endpoints were presented based on the mean measured concentrations which accounts for the loss of test material and provides a reflection of the toxicity of the parent plus the metabolites formed. This RMS has performed a statistical analysis following OECD 201 recommendations: a logistic regression has been applied (LogLogistic 4 parameters model), the regression analysis have been performed using individual replicate responses, not treatment group means, the goodness of fit of the response data to the regression model has been assessed graphically and statistically. Considering the geometric mean measured concentrations (with MQL/2 = 0.00535 mg/L):
ErC_{10} = 0.021 mg/L (c.i. 0.019 – 0.024)
ErC_{50} = 0.035 mg/L (c.i. 0.028 - 0.039)

A further OECD 201 study with *Desmodesmus subspicatus* was conducted ([redacted] 1998; A7.4.1.3_02), in which algistatic and algicidal effects were additionally investigated according to US EPA OPPTS 850.5400 “Algal Toxicity”, Tiers I and II. The algistatic effect is defined as cell growth inhibition with cells still surviving whereas the algicidal effect refers to cell death. Due to these additional endpoints the test duration was prolonged to 7 days, but as the E_rC_{50} values were determined after 72 and 96 hours, the results could still be compared with those of the remaining tests. The concentrations were measured during the test, but they are based on the sum of two peaks (nominals 0.032, 0.1, 0.32, 1.0, 3.2, 10 mg/L and the geomean concentrations are 0.037, 0.116, 0.238, 0.76, 3.05 and 9.305 respectively). An algicidal effect of Bronopol was indicated at 3.2 mg/L (nominal) and higher test concentrations. Recovery of algal cells treated with up to nominal 1.0 mg/L test substance was observed if the algal cells were incubated in culture medium without test substance for some days. Analytical dose verification of the test substance displayed recovery rates from 37 to 89 % of the nominal values, whereas the highest recovery rates were observed in the lowest and highest test concentrations (75% for 0.032 mg/L, 86% for 3.2 mg/L and 89% for 10 mg/L). Due to the low recoveries, the data should be considered carefully. The areas taken for the analytical determination correspond to two different peaks (at 7,5-7,7 and 11,5-11,9 minutes, hence the parent and at least one main metabolite are present). As initial measured concentrations are 95-125% of nominals, they could be used for endpoint derivation. Nevertheless, the statistical analysis performed showed very wide confidence intervals for ECx derivation, including zero value for EC10, hence this study is not suitable for deriving such endpoints.

For the freshwater compartment not only toxicity towards green algae was tested but also toxicity towards cyanobacteria (representative species: *Anabaena flos-aquae*) and diatoms (representative species: *Navicula pelliculosa*) by [redacted] (2006b (A7_4_1_3-02) and 2006c (A7_4_1_3-04)). In the test with *Navicula*, the EC50 values and corresponding 95% confidence intervals for cell density, area under the growth curve and growth rate for each 24-hour exposure period were calculated (where possible) using non-linear regression or linear interpolation. The data were evaluated for normality and homogeneity of variance (p=0.05) using Shapiro-Wilk’s and Levene’s tests, respectively. The NOEC values were determined by comparison of the treatment groups to the control using Dunnett’s test (p=0.05). While *N. pelliculosa* in the study of [redacted] (2006c; A7_4_1_3-04) was less sensitive than the green algae (72h-E_rC_{50} = 0.459 mg/L, initial measured and 0.135 as geometric mean measured concentration and a NOEC = 0.212 mg/L or 0.065 mg/L as a geomean), *A. flos-aquae* in the study of [redacted] (2006b; A7_4_1_3-02) was more sensitive than most of the tested green algae (72h-E_rC_{50} = 0.068 mg/L, initial measured or 0.019 as geometric mean). Nevertheless, in this test with *Anabaena*, the cell growth curve is not accurate at the two higher concentrations and the test does not meet validity criterion 3 of 10% coefficient variation 0-72h in the control for less frequent species; further, replicates show a cell density of 0 at 24 h in several replicates at different concentrations.

Hence, the test is considered only as supporting information.

Only for *Desmodesmus subspicatus* a lower 72h-E_rC_{50} value was determined in the study of [redacted] (2006a; A7_4_1_3-01). Based on the result of this study *D. subspicatus* was considered as most sensitive algal species and thus the 72h-E_rC_{50} of 0.0073 mg/L based on geomean is the acute endpoint selected.

For the saltwater compartment two studies were performed, both on the marine diatom *Skeletonema costatum* ([redacted] 2006d (A7_4_1_3-05) and [redacted] 1998 (A7.4.1.3_03)). The growth inhibition test of [redacted] (1998; A7.4.1.3_03) was conducted according to ISO guideline 10253 and algistatic and algicidal effects were additionally investigated according to US EPA OPPTS 850.5400 “Algal Toxicity”, Tiers I and II. For the assessment of these additional parameters, the test duration was prolonged to 7 days, but the EC_{50} values were determined for the standard time period of 72 hours. Since no analytical monitoring was performed, the results were based on nominal concentrations giving a 72h-E_rC_{50} of 0.25 mg/L. This value is comparable to the 72h-E_rC_{50} of 0.178 mg/L determined based on initial measured concentrations in the second study of [redacted] (2006d; A7_4_1_3-05), for comparability. In the test from [redacted] (2006d; A7_4_1_3-05), algal density at the start of the tests was 70,000 cells per mL, as recommended in the US EPA OPPTS 850.5400 guideline. At 72 hours, Bronopol had no significant effect on algal growth in the three lowest test concentrations (12, 23, and 52 µg/L nominal) for the cell density and growth rate endpoints. There was clear dose-response demonstrated over the range of test concentrations with approximately 90% inhibition of density at the highest test concentration. All treatment concentrations were significantly less than the controls for area under the growth curve at 72 hours. For cell density and growth rate, the same conclusions can be drawn at 96 hours as for 72 hours. Only the lowest treatment level was not significantly less than the controls for area under the curve at 96 hours. As with the 72-hour endpoints, a strong dose-response was still seen over the highest remaining test levels. Based on geometric mean concentrations: 72h-NOErC (growth rate) = 0.015 mg/L and 72h-ErC50 (growth rate) = 0.052 mg/L.

Bronopol showed a high acute toxicity to algae, indicating that algae were the most sensitive species within the group of aquatic organisms tested. Among the different algal species diatoms were slightly less susceptible to Bronopol than green algae and cyanobacteria, with no significant difference between freshwater and marine species. In contrast, aquatic plants represented by the floating species *Lemna gibba* were found to be the least sensitive group of aquatic organisms used in the tests at all.

Information for metabolites of Bronopol

For the degradation products tris(hydroxymethyl)nitromethane (TNM) and 2-bromo-2-nitroethanol, the following ecotoxicological data for the three standard species were generated:

Test species	Test substance	Result
Rainbow trout (*Onchorynchus mykiss*)	Tris(hydroxymethyl)nitromethane	410 mg/L (96h-LC_{50})
	2-bromo-2-nitroethanol	3 mg/L (96h-LC_{50})
Water flea (*Daphnia magna)*	Tris(hydroxymethyl)nitromethane	80 mg/L (48h-EC_{50})
	2-bromo-2-nitroethanol	0.38 mg/L (48h-EC_{50})
Green algae (*Raphidocelis subcapitata)*	Tris(hydroxymethyl)nitromethane	>4.5 mg/L (72h-EC_{50})
	2-bromo-2-nitroethanol	0.019 mg/L (72h-EC_{50}) based on TWA

The test with TNM ([redacted] 2002; A7_4_1_3-06) with algae, meet the validity criteria but has shown some deficiencies in the results obtained from the statistical analysis (Rstats): lack of normality is present in the data, and the calculations include a 0 as a possible EC50, which is not a consistent result). The assay should have included higher concentrations to those used in the available assay, in order to obtain an adequate sigmoid dose-response curve. The mean measured concentrations were 0.0172, 0.0422, 0.109, 0.269, 0.628, 1.61, and 4.5 mg/L for the 0.0200, 0.0500, 0.130, 0.320, 0.800, 2.00, and 5.00 mg/L nominal concentrations, respectively. Hence the EC50 based on measured concentrations is > 4.5 mg/L and the 72-hour NOEC is 0.269 mg/L.

The algae test with 2BNE ([redacted] 2012; A7_4_1_3-08), met the validity criteria in OECD TG 201. The results are based on time-weighted average concentrations of 2-bromo-2-nitroethanol and are reported as the 72-hour EC10, EC20 and EC50 values for biomass expressed as yield and average growth rate data calculated from the 72-hour cell density counts, and the NOEC values for total yield and average growth rate. After 72 hours, the TWA mean concentrations were 0.0052, 0.012, 0.034, 0.11, 0.30 and 0.89 mg a.i./L, hence the concentration of test substance is not ≥ 80% of initial concentration during test. The applicants have stated that the TWA approach was used for the calculation of the test item concentrations because the sampling intervals were not equally spaced (the test solutions were sampled and analysed at t=0, 24 and 72 hours, no sampling at t=48 hours). According to the study plan, the initially planned sampling points were t=0 and 72 hours, i.e., at start and end of exposure period. Additional sampling after 24 hours was planned in case of dissipation of test item (judgement by study director). This is in line with OECD TG 201, paragraph 37 “For volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24-hour intervals during the exposure period are recommended in order to better define loss of the test substance.” Additionally, in the OECD GD 23 for difficult test chemicals it is stated in paragraph 176 that “If measured concentrations in samples do not remain within 80-120% of nominal, the effect concentration should be expressed relative to the measured concentrations. In this situation, (…), effects concentrations may be determined and expressed relative to the time-weighted mean measured concentrations.” Regarding the statistical analysis performed by this eCA, we found some issues with the normality at the lowest and highest concentrations, which might be related to the rapid disappearance of 2BNE. Based on a statistical analysis from this eCA, based on TWA concentrations, the EC10 (72h, growth rate) is 0.019 mg/L (0.007-0.03); EC50 (72h, growth rate) = 0.109 mg/L (0.054-0.164).

These data show that TNM is of significantly lower toxicity to aquatic species compared to Bronopol, while for BNE a similar but a little higher level of toxicity to aquatic species was observed. The disappearance during the course of the biodegradation studies with each of both metabolites demonstrates the transient nature of the metabolites. Moreover, BNE is considered to degrade very rapidly in the STP, according to an OECD 314 simulation test.

For aquatic plant *Lemna gibba* a test was conducted ([redacted], 2006e; A7_4_3_5_2-01). The initial (day 0) measured test concentrations were <LOQ, 6.84, 13.8, 27.0, 54.5, 110, 221 and 442 mg/L, and at test termination <LOQ, 4.04, 9.70, 20.7, 46.1, 98.4, 209 and 423 mg Bronopol/L were found. Geometric mean between time zero and time 7 days was calculated. There is not much loss of Bronopol in this test because it is stabilized with methanol. Number of fronds at the start of the test was 12 fronds/4 plants per replicate. After 7 days, Bronopol exerted no significant effect on duckweed growth in the two lowest test concentrations, whilst growth was almost completely inhibited in highest test concentrations. Concentration-dependent decrease in growth was found for the medium test concentrations. At concentrations of nominal 13 mg/L and above, treatment-related effects on fronds and plants were evident, including small fronds, root destruction, curled frons and/or breakup of colonies in comparison to the control. The NOEC is 5.4 mg/L based on biomass (geometric mean) and the EbC50 / ErC50 are 39 mg/L / 142 mg/L (EC50 frond number: 87 mg/L) (geometric mean).

Chronic / long-term toxicity (freshwater)

Table A-47: Summary table - chronic/long-term aquatic toxicity

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LOEC/NOEC/EC_{10} (specify the value)		
Fish								
OECD 215 (2000), GLP, Rel. 1 Key	Rainbow trout, *Oncorhynchus mykiss*	Mortality, growth / long-term	Bronopol	Flow-through	28 days	NOEC=2.57 mg a.s./L LOEC=9.3 mg a.s./L	Dose-response, mean measured conc.	[redacted] 2007 (A7_4_3_1-01)
OECD 210 (1984), GLP, Rel. 2	*Oncorhynchus mykiss*	mortality	Bronopol	Flow-through	49 days	NOEC = 1.74 mg/l (measured) LOEC = 7.09 mg/l (measured) EC50 = 4.508 mg/l (measured, limits: 2.165 – 3.751 mg/l)	Measured concentrations	[redacted] 1996 (A7.4.3.2_01)
Invertebrates								
OECD 211 (1998), GLP, Rel. 2 Key	Water flea, *Daphnia magna*	Survival, reproduction, growth / chronic	Bronopol	Flow-through	21 days	NOEC=0.058 mg a.s./L LOEC=0.109 mg a.s./L	Dose-response, mean measured conc.	[redacted] 2004 (A7_4_3_4-01)
OECD 202 (1984), GLP, Rel. 2	Daphnia magna	Mortality, number and condition of newborn	Bronopol	Flow-through	21 days	NOEC=0.029 mg/l (measured)	No initial measured value exists for NOEC as this concentration was only measured after 7 days for the first time.	[redacted] 1992 (A7.4.3.4_01)
Algae[1]								
OECD 201 (1984), 92/69/EEC Method	Freshwater green microalga,	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.0026 mg/L72h-	geometric mean measured concentration	[redacted] 2006a

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
C.3 (1992), US EPA OPPTS 850.5400 (1996), GLP, Rel. 2 Key	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)					EC10=0.0048 mg/L, calculated and used for PNEC derivation)		(A7_4_1_3-01)
OECD 201 (1984), GLP, Rel. 3	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.10 mg/L	Nominal conc., no analytical monitoring	[redacted] 1994 (A7.4.1.3_01c)
OECD 201 (1984), US EPA OPPTS 850.5400, Rel. 2 GLP	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	growth inhibition, Algistatic effects	Bronopol	static	96 hours plus 7 days recovery period	72h-NOEC=0.10 mg/L	Nominal conc.	[redacted] 1998 (A7.4.1.3_02)
OECD 201 (1984), 92/69/EEC Method C.3, US EPA OPPTS 850.5400, Rel. 3 GLP	Freshwater bluegreen alga (cyanobacteria), *Anabaena flos-aquae*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.047 mg/L (initial) and 0.016 (geomean)	measured concentration	[redacted] 2006b (A7_4_1_3-02)
OECD 201 (1984), US EPA OPPTS 850.5400, GLP, Rel. 2	Freshwater green microalga, *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.0188 mg/L EC10 = 0.021 mg/L (calculated based on geometric mean measured)	measured concentration	[redacted] 2002 (A_7_4_1_3-03)
OECD 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (formerly *Pseudokirchneriella*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.10 mg/L	Nominal conc., no analytical monitoring	[redacted] 1994 (A7.4.1.3_01a)

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
	subcapitata and *Selenastrum capricornutum*)							
OECD 201 (1984), 92/69/EEC Method C.3, US EPA OPPTS 850.5400, Rel. 2 GLP	Freshwater diatom, *Navicula pelliculosa*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.212 mg/L (initial) and 0.065 mg/L (geomean)	measured concentration	[redacted] 2006c (A7_4_1_3-04)
OECD 201 (1984), GLP, Rel. 3	Freshwater green microalga, *Chlorella vulgaris*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.32 mg/L	Nominal conc. due to inconsistencies in analytical measurement	[redacted] 1994 (A7.4.1.3_01b)
OECD 201 (1984), US EPA OPPTS 850.5400, Rel. 2 GLP	Marine diatom, *Skeletonema costatum*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.015 mg/L	Geometric mean measured concentration	[redacted] 2006d (A7_4_1_3-05)
ISO 10253, US EPA OPPTS 850.5400, Rel. 2 GLP	*Skeletonema costatum*	growth inhibition Algistatic effects	Bronopol	static	72 hours plus 7 days recovery period	72h-NOEC=0.08 mg/L	Nominal conc., no analytical monitoring	[redacted] 1998 (A7.4.1.3_03)
OECD 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	Growth inhibition	2-bromo-2-nitroethanol (BNE)	static	72 hours	72h-E_rC_{10} = 0.019 mg/L	Time-weighted average, mean measured conc.	[redacted] 2012 (A7_4_1_3-08)
OECD 201 (1984), Directive 92/69/EEC Method C.3, GLP, Rel. 2	*Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	Growth inhibition	Tris-hydroxymethyl-nitromethane (Tris Nitro, TNM)	static	96 hours	E_rC_{10} = 0.572 mg/L	Mean measured conc.	[redacted] 2002 (A7_4_1_3-06)

Method, Guideline, GLP status, Reliability, Key / supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results	Remarks	Reference
Other aquatic plants								
OECD 221 (proposal), GLP, Rel. 1	Freshwater duckweed, *Lemna gibba*	frond growth	Bronopol	static	7 days	NOEC=5.4 mg a.s./L	Dose-response, mean measured conc.	[redacted] 2006e (A7_4_3_5_2-01)

[1] calculated from growth rate, if not available please include the biomass value (NOE_bC/E_bCx) or the unspecified NOEC/ECx value.

Description of the available chronic toxicity studies

Chronic toxicity to fish

Long-term effects of Bronopol to fish (rainbow trout *Oncorhynchus mykiss*) were investigated in a 28-day juvenile growth test according to OECD TG 215 ([redacted] 2007; A7_4_3_1-01) and a 49-day flow-through test according to OECD TG 210 ([redacted] 1996; A7.4.3.2_01). The observed test parameters for the concentration ranges of nominal 0.32 to 32 mg/L ([redacted] 2007; A7_4_3_1-01) and 2.25 to 40 mg/L ([redacted] 1996; A7.4.3.2_01) were mortality, sublethal effects, and body weight.

In the study of [redacted] (2007 A7_4_3_1-01) juvenile trout exposed to 9.3 mg/L (mean measured conc., LOEC) exhibited slight increase in mortality in comparison to the control, whilst neither lethal nor sublethal effects occurred in the next lower test concentration of 2.6 mg/L (mean measured conc.) which was therefore defined as NOEC. The test concentrations below the NOEC were not analysed, for the remaining 3 concentrations (NOEC, LOEC and highest concentration) recoveries between 82% and 94% were achieved. Bronopol was demonstrated to be sufficiently stable in the test medium during the test period, except the day 3, 7 and 21 measurements at the 3.2 mg a.s./L treatment level showing 54, 78 and 74% of nominal, respectively. For the rest of the measurements, recovery of Bronopol was always >80% of nominal.

The chronic toxicity of Bronopol to rainbow trout (*Oncorhynchus mykiss*) was investigated in a 49-day flow-through test according to OECD guideline 210 ([redacted], 1996; A7.4.3.2_01). The nominal test concentrations were 2.25, 4.41, 6.85, 11.46, 21.48 and 40.0 mg/L Bronopol. Test parameters observed were mortality, sublethal effects, body length, body weight, and condition indices (CI). Survival of early-life-stage rainbow trout was significantly affected by a chronic treatment at nominal test concentrations of 40 mg/L. Mortality for all other test concentrations (2.25 to 21.50 mg/L) was within the control range. For the control, a mortality of 11.25% was reported, which was in accordance with the validity criteria of OECD TG 210 requesting a hatching success >66% and a post-hatch survival of 70% for the control group. None of the treated groups differed significantly from the control in terms of body weight, length and condition indices. The resulting NOEC based on mortality was nominal 21.5 mg/L. Analytical monitoring of the test substance revealed a recovery rate between 1.6 and 19.5% of the nominal concentrations. The test is acceptable, but the results should be considered carefully due to such a low recovery rate. A comparison to reference standards showed that at elevated pH values (pH 8.16 to 8.23) and under aerobic conditions, Bronopol seems to preferentially degrade to 2-bromo-2-nitroethanol (BNE). The rapid transformation of Bronopol into different metabolites indicates that the toxicity observed from long exposure test should be considered as toxicity caused by a mixture of compounds.

Chronic toxicity to aquatic invertebrates

Long-term effects of Bronopol to invertebrates (daphnids) were investigated in two chronic reproduction tests, exposing the test organisms under flow-through conditions to concentrations ranging from nominal 0.025 to 0.400 mg/L ([redacted] 2004; A7_4_3_4-01) and nominal 0.017 to 1.7 mg/L ([redacted] 1992; A7.4.3.4_01) Bronopol for 21 days. Test parameters were immobility of parental daphnids, as well as total body length of surviving adult daphnids on day 21, and number and condition of newborn daphnids/offspring.

In the chronic *Daphnia* test according to OECD TG 211 by [redacted] (2004; A7_4_3_4-01), reproductive efficiency and growth of adult daphnids were the most sensitive parameters, both significantly reduced in the highest test concentration of 0.110 mg/L (mean measured, LOEC) in comparison with the control, whilst survival was not affected at this concentration level. No statistically significant differences to the control group were found at the next lower concentration of 0.058 mg/L (geometric mean measured), which was

therefore defined as NOEC. Recoveries were in a range of 18% to 30% and were explained by the hydrolysis of Bronopol in water which runs faster than the replenishment via the diluter system.

In the *Daphnia magna* reproduction test according to OECD TG 202 (1984) ([redacted] 1992; A7.4.3.4_01), 100% mortality was observed at the highest concentration (nominal 1.7 mg/L), whereas in all other treatments, mortality was <20% and therefore within the control range. As the treatment with the highest test concentration was subsequently deleted from the concentration range, and further, as the mean number of live offspring produced per parent control animal was ≥60, the test can be considered as valid.

For all tested concentrations (except for the highest test concentration of nominal 1.7 mg/L where 100% parent mortality occurred), no adverse effects on reproduction could be detected. In fact, from Day 9 of exposure a statistically significant stimulation of reproduction was noted. Hence, the second highest test concentration of 0.53 mg/L (nominal) was defined as NOEC (0.029 as mean measured concentration). Results should be expressed as mean measured or at least as initial measured, but there is no analytical determination for this test concentration at time 0. The active substance disappears very rapidly in the test system; hence, these results should be considered carefully.

Analytical monitoring of the test substance showed a recovery rate ranging from 35% to 52% of nominal concentrations. At the end of the test, the recovery rate was about 13%. Since Bronopol is known to hydrolyse rapidly at pH values above 5 and the guideline provides a pH range of 7.8 to 8.3 for the test, the hydrolysis mechanism might be responsible for the low analytical recovery rate and could not be compensated by an increase of the flow rate either.

Chronic toxicity to algae or other aquatic plants

Chronic effects of Bronopol to algae and aquatic macrophytes were investigated for several freshwater and one marine species already explained for acute toxicity section. Algae were clearly the most sensitive group of the primary producers with the lowest NOEC of 0.0052 mg a.s./L or EC10 of 0.0125 mg/L (based on initial measured concentration), determined for *D. subspicatus* in the OECD 201 study of [redacted] (2006a; A7_4_1_3-01). Cyanobacteria and marine diatoms were slightly less susceptible to Bronopol than green algae, whilst aquatic plants represented by the floating species *L. gibba* were found to be the least sensitive group of aquatic organisms used in the tests at all.

A.4.2.3.2 Sediment compartment (freshwater)

Acute/short-term toxicity (freshwater sediment)

Data waiving	
Information requirement	Studies on sediment-dwelling organisms (BPR Annex II, Title 1, Point 9.1.9.)
Justification	Based on the trigger value given in Guidance on BPR vol. IV Part B+C, chapter 3.5.2 (log Koc or log Kow ≥3), a sediment effect assessment is not required for Bronopol as the trigger value is not met for this active substance.

Chronic/long-term toxicity (freshwater sediment)

Data waiving	
Information requirement	Studies on sediment-dwelling organisms (BPR Annex II, Title 1, Point 9.1.9.)
Justification	Based on the trigger value given in Guidance on BPR vol. IV Part B+C, chapter 3.5.2 (log Koc or log Kow ≥3), a sediment effect assessment is not required for Bronopol as the trigger value is not met for this active substance.

A.4.2.3.3 Marine compartment

Acute/short-term toxicity (seawater)

Data waiving	
Information requirement	Not specified in BPR Annex II
Justification	According to the use patterns of Bronopol (PT 2, 6, 11 and 12), exposure to seawater is unlikely.

Chronic/long-term toxicity (seawater)

Data waiving	
Information requirement	Not specified in BPR Annex II
Justification	According to the use patterns of Bronopol (PT 2, 6, 11 and 12), exposure to seawater is unlikely.

A.4.2.3.4 Seawater sediment compartment

Acute/short-term toxicity (seawater sediment)

Data waiving	
Information requirement	Not specified in BPR Annex II
Justification	According to the use patterns of Bronopol (PT 2, 6, 11 and 12), exposure to sea sediment is unlikely.

Chronic/long-term toxicity (sea sediment)

Data waiving	
Information requirement	Not specified in BPR Annex II
Justification	According to the use patterns of Bronopol (PT 2, 6, 11 and 12), exposure to sea sediment is unlikely.

A.4.2.3.5 Higher tier studies on aquatic organisms

No data available.

A.4.2.4 Terrestrial compartment

Not applicable for the CLH report.

A.4.2.5 Groundwater

Not applicable for the CLH report.

A.4.2.6 Birds and mammals

Not applicable for the CLH report.

A.4.2.7 Primary and secondary poisoning

Not applicable for the CLH report.

A.4.3 Endocrine disruption

Not applicable for the CLH report.

A.4.4 Derivation of PNECs

Not applicable for the CLH report.

A.4.5 Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

A.4.5.1 Acute aquatic hazard

Table A-48: Summary of key information on acute/ short-term aquatic toxicity relevant for aquatic acute classification

Method	Species	Test material	Results[1]	Remarks	Reference
Fish					
No relevant data for classification, 96h-LC_{50} in all studies on Bronopol > 1 mg/L					
Invertebrates					
No relevant data for classification, 48h-EC_{50} in all studies on Bronopol > 1 mg/L					
Algae					
OECD 201 (1984), 92/69/EEC Method C.3 (1992), US EPA OPPTS 850.5400 (1996)	Freshwater green microalga, *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	Bronopol	72h-E_rC_{50} = 0.0073 mg/L	GLP study, results based on geometric mean measured concentration	[redacted] 2006a (A7_4_1_3-01)
Other aquatic plants					
No relevant data for classification, 7d-EC_{50} in the *Lemna* study on Bronopol > 1 mg/L					

[1] Indicate if the results are based on (initial or mean) measured or on the nominal concentrations and/or any information on degradants/ metabolites.

According to CLP criteria, Annex I: Table 4.1.0 “Classification categories for hazardous to the aquatic environment”, Bronopol is considered to fulfil classification as short-term (acute) aquatic hazard Category Acute 1 as ErC50 at 72 h = 0.0073 mg/L for algae is < 1 mg/l.

A.4.5.2 Long-term aquatic hazard (including information on bioaccumulation and degradation)

Table A-49: Summary of key information on chronic/ long-term aquatic toxicity relevant for aquatic chronic classification

Method	Species	Test material	Results[1]	Remarks	Reference
Fish					
No relevant data for classification, 28d-NOEC in chronic fish study on Bronopol > 1 mg/L					
Invertebrates					
OECD 211 (1998)	Water flea, *Daphnia magna*	Bronopol	NOEC=0.06 mg a.s./L	GLP study, results based on mean measured conc.	[redacted] 2004 (A7_4_3_4-01)
Algae					
OECD 201 (1984), 92/69/EEC Method C.3 (1992), US EPA OPPTS 850.5400 (1996)	Freshwater green microalga, *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	Bronopol	72h-EC10=0.0048 mg/L	GLP study, results based on geometric mean measured concentration	[redacted] 2006a (A7_4_1_3-01)
Other aquatic plants					
No relevant data for classification, 7d-NOEC in the *Lemna* study on Bronopol > 1 mg/L					

[1] Indicate if the results are based on (initial or mean) measured or on the nominal concentrations and/or any information on degradants/ metabolites.

Potential on bioaccumulation

The n-octanol/water partition coefficient (Pow) of Bronopol is 0.48, 0.38 and 0.31 (log Pow -0.32, -0.42, -0.50) at 10, 20 and 30°C, respectively. Therefore, the substance is considered to have a negligible potential for bioconcentration due to its lack of lipophilicity. In general, for substances with a log Pow < 3 the experimental determination of the BCF is not required. Furthermore, Bronopol is not surface active (surface tension of 72 mN/m at a concentration of 1.0 g/L).

This assumption is also supported by the high water solubility of Bronopol, i.e., 304 g/L at 20°C, limiting the substance's affinity to partition to lipid compartments and thus the probability of bioconcentration.

QSAR modelling also provides a very low Log Octanol-Water Partition coefficient (SRC): Log Kow (KOWWIN v1.67 estimate) = -0.64

For the main transient product, BNE, QSAR modelling provides as well very low Log Kow (KOWWIN v1.67 estimate) = -0.74 and for the main metabolite, TNM, Log Kow (KOWWIN v1.68 estimate) = -1.66.

Hence, no bioaccumulation is expected.

In case of soil organisms, Bronopol is expected to remain in the pore water based on the high water solubility and the low log Pow.

Abiotic degradation

Both the two valid studies for hydrolysis of Bronopol and the publications available, demonstrate that the hydrolysis of Bronopol takes place very rapidly at environmental relevant pH values, with a significant pH and concentration dependency (accelerated rate of hydrolysis at lower concentrations and elevated pH). In most of the possible degradation pathways, a transient product, 2-bromo-2-nitro-ethanol (BNE, whose concentration remained constant in equilibrium with the parent) if formed. Other degradation pathways lead to the possible formation of Tris(hydroxymethyl)nitromethane (TNM) as main metabolite.

Regarding photolysis, with a half-live of 20 days, under natural conditions, hydrolysis and primary biodegradation in water are expected to be more rapid than photolysis of Bronopol. This is especially true taking into account that sunlight penetrates only the very uppermost layers of natural waters. Therefore, potential phototransformation products of Bronopol are expected to occur only at negligible levels in the environment.

Bronopol has a very low Henry´s Law constant and therefore volatilisation is not to be expected. The rate constant for phototransformation of Bronopol in air was estimated using the AOPWIN software showing a slow degradation in air.

Biotic degradation

Bronopol's potential of readily biodegradation was investigated in several studies according to different methods described in the OECD guideline 301. The classification target of 60% mineralisation is not met and Bronopol can thus not be classified as readily biodegradable.

While the criteria for readily biodegradability was not fulfilled in neither of these studies, signs of abiotic degradation (e.g. hydrolysis) were observed indicating that abiotic degradation processes are predominant under the respective test conditions and thus at environmental relevant pH values.

Bronopol is considered as not rapidly degradable for classification purposes because:

a. It is not readily biodegradable in the ready biodegradability tests.

b. There is not an available surface water simulation test to demonstrate a half-life of < 16 days (corresponding to a degradation of >70 % within 28 days).

c. Although the substance is demonstrated to be primarily degraded abiotically e.g. via hydrolysis, in the aquatic environment with a half-life <16 days (corresponding to a degradation of >70 % within 28 days), it cannot be demonstrated that the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment. BNE as intermediate product, is toxic to the environment and fulfil the criteria for classification as hazardous to the aquatic environment.

Hence, according to CLP criteria, Annex I: Table 4.1.0 “Classification categories for hazardous to the aquatic environment”, Bronopol is considered to fulfil classification as long-term (chronic) aquatic hazard Category Chronic 1 as a non-rapidly degradable substances for which there are adequate chronic toxicity data available, with a chronic EC_{10} for algae = 0.0048 < 0,1 mg/L.

A.4.5.3 Conclusion on classification and labelling for environmental hazards and comparison with the CLP criteria

Aquatic Acute

According to Annex I: Table 4.1.0 “Classification categories for hazardous to the aquatic environment”, Bronopol is considered to fulfil classification as short-term (acute) aquatic hazard Category Acute 1 as ErC50 = 0.0073 mg/L for algae is < 1 mg/l. Being 0,001 < L(E)C50 < 0,01 mg/L, according to Annex I: Table 4.1.3, the multiplying factor is M = 100.

Aquatic Chronic

According to Annex I: Table 4.1.0 “Classification categories for hazardous to the aquatic environment”, Bronopol is considered to fulfil classification as long-term (chronic) aquatic hazard Category Chronic 1 as a non-rapidly degradable substances for which there are adequate chronic toxicity data available, with a chronic EC_{10} for algae = 0.0048 < 0,1 mg/L.

Being 0,001 < NOEC/EC10 < 0,01 mg/L, according to Annex I: Table 4.1.3, the multiplying factor is M = 10.

A.5 Assessment of additional hazards

A.5.1 Hazardous to the ozone layer

Not applicable.

A.5.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not applicable.

A.5.1.2 Comparison with the CLP criteria

Not applicable.

A.6 Additional Labelling

Not relevant.

A.7 Assessment of exclusion criteria, substitution criteria and POP

Not applicable for the CLH report.

D. Appendices

Appendix V: Overall reference list (including data owner and confidentiality claim)

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
	2001	A 3.2.1	Bronopol: Acute oral toxicity study in Fischer 344 rats; [redacted], The Dow Chemical Company, Midland, Michigan, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2000	A 3.2.2	Bronopol Bayer: Acute dermal toxicity study in male and female Wistar rats; [redacted], Bayer AG, Wuppertal, Germany; report number [redacted] (study No [redacted]), (including amendment); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2003	A 3.2.3	Revised report for: Bronopol: Acute dust aerosol inhalation toxicity study in Fisher 344 rats; [redacted], The Dow Chemical Company, Midland, Michigan, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2000a	A 3.3	Acute skin irritation test (patch test) of Bronopol Bayer in rabbits; [redacted], Hamburg, Germany; Report No [redacted] (LPT Report No [redacted], Bayer Study No [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2000b	A 3.4	Acute eye irritation study of Bronopol Bayer by instillation into the conjunctival sac of one rabbit; [redacted], Hamburg, Germany; Report No [redacted] (LPT Report No [redacted], Bayer Study No [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2001	A 3.5	Bronopol Bayer: Study for the skin sensitisation effect in Guinea pigs (Guinea pig maximization test according to Magnusson and Kligman); [redacted], Bayer AG, Wuppertal, Germany; Report No [redacted] (study No [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
	2005	A 3.5	Bronopol: Local lymph node assay in BALB/cAnNCrl mice; [redacted], The Dow Chemical Company, Midland, Michigan, USA; Report No [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
Maibach, H.I.	1977	A 3.3 A 3.5	Dermal sensitization potential of 2-bromo-2-nitropropane-1,3-diol (Bronopol); Contact Dermatitis, 1977, 3:99-108; Published	No	-
Peters, M.S., Connolly S.M., Schroeter, A.L.	1983	A 3.3 A 3.5	Bronopol allergic contact dermatitis; Contact Dermatitis, 1983, 9:397-401; Published	No	-
Schnuch, A., Geier, J., Uter, W., Frosch P.J.	1998		Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study; British Journal of Dermatology, 1998, 138:467-476; Published	No	-
Shaw, S.	1997	A 3.5	Patch testing Bronopol – Defining the optimal conditions for getting accurate readings on the allergenicity of Bronopol; Cosmetics & Toiletries Magazine, 1997, 112:67-73; Published	No	-
Britton, J.E.R., Wilkinson, S.M., English, J.S.C., Gawkrodger, D.J., Ormerod, A.D., Sansom, J.E., Shaw, S., Statham, B.	2003	A 3.5	The British standard series of contact dermatitis allergens: validation in clinical practice and value for clinical governance; British Journal of Dermatology, 2003, 148:259-264; Published	No	-
Camarasa, J.G.	1986	A 3.5	Contact dermatitis due to Bronopol; Contact Dermatitis, 1986, 14:191-192; Published	No	-
Frosch, P.J., Weickel, R.	1987	A 3.5	Kontaktallergie auf das Konservieringsmittel Bronopol; Hautarzt, 1987, 38:267-270; Published	No	-
Frosch, P.J., White I.R., Rycroft R.J.G., Lahti, A., Burrows D., Camarasa, J.G., Ducombs, G., Wilkinson, J.D.	1990	A 3.5	Contact allergy to Bronopol; Contact Dermatitis, 1990, 22:24-26; Published	No	-
Koch, C.S.	2000	A 3.5	The safety of biocides for cosmetics and toiletries; Biocides Today, 2000, 13-14; Published	No	-
Marzulli, F.N., Maibach, H.I.	1973	A 3.5	Antimicrobials: Experimental contact sensitization in man; J Soc Cosmet Chem, 1973, 24:399-421; Published	No	-

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
Marzulli, F.N., Maibach, H.I.	1974	A 3.5	The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man; Fd Cosmet Toxicol, 1974, 12:219-227; Published	No	-
Podmore, P.	2000	A 3.5	Occupational allergic contact dermatitis from both 2-bromo-2-nitropropane-1,3-diol and methylchloroisothiazolinone plus methylisothiazolinone in spin finish; Contact Dermatitis, 2000, 43: 45; Published	No	-
Schlede, E., Aberer, W., Fuchs, T., Gerner, I., Lessmann, H., Maurer, T., Rossbacher, R., Stropp, G., Wagner, E., Kayser, D.	2004	A 3.5	Chemische Substanzen und Kontaktallergie – eine Bewertung von 244 Substanzen; Dermatologie in Beruf und Umwelt, 2004, 4:146-163; Published	No	-
Kujawa, M., Macholz, R., Seidler, H., Härtig, M., Lewerenz, H.J., Schnaak, W., Zydek, G.	1987	A 3.1	Verteilung und Metabolismus von 2-Brom-2-nitropropan-1,3-diol (Bronopol); Z gesamte Hyg, 1987, 33 (Heft 1): 27-29; Published	No	-
Moore, D.H., Chasseaud, L.F., Lewis, J.D., Risdall, P.C., Crampton, E.L.	1976	A 3.1	The metabolism of the antibacterial agent Bronopol (2-bromo-2-nitropropane-1,3-diol) given orally to rats and dogs; Fd Cosmet Toxicol, 1976, 14:183-187; Published	No	-
Moore, D.H., Chasseaud, L.F., Bucke, D., Risdall, P.C.	1976	A 3.1	The percutaneous absorption and disposition of the antibacterial agent Bronopol in rats and rabbits; Fd Cosmet Toxicol, 1976, 14:189-192; Published	No	-
Buttar, H.S., Downie, R.H.	1980	A 3.1	The biotransformation and disposition of Bronopol following topical and intravenous administration to rats; Toxicology Letters, 1980, 6:101-107; Published	No	-
	2007	A 3.1	Bronopol: Oral absorption, distribution and elimination in Crl:CD(SD)-Sprague Dawley derived rats; [redacted], The Dow Chemical Company, Midland, Michigan, USA; report number [redacted] (Study [redacted]); GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006	A 3.7.1.1	Water palatability study in Beagle dogs; [redacted], Kansas City, Missouri, USA; MRI Project No	Yes	Nutrition & Biosciences (Switzerland)

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			310358.1.140.01 (report number [redacted]); Unpublished		GmbH, LANXESS Deutschland GmbH
	2006	A 3.7.1.1	Bronopol: 28-day drinking water toxicity study in Beagle dogs; [redacted], Mattawan, Michigan, USA; Study number 133-070 (report number [redacted]); GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2001	A 3.7.2.1	Bronopol Bayer 2-Brom-2-nitropropane-1,3-diol: Study for subchronic oral toxicity in rats (drinking water study over 13 weeks and 4 weeks recovery); [redacted], Bayer AG, Wuppertal, Germany; report number [redacted] (study No [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2006	A 3.7.2.1	Bronopol: 90-day drinking water toxicity probe study in CRL:CD(SD) rats; [redacted], The Dow Chemical Company, Midland, Michigan, USA; Study number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2007	A 3.7.2.1	Bronopol: 90-day drinking water toxicity study in Beagle dogs; [redacted], Mattawan, Michigan, USA; study number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
Hunter, B., Batham, P., Heywood, R.	1976	A 3.7.3.1 A 3.9	Bronopol toxicity and tumorigenicity study in rats by administration in the drinking water for 104 weeks[1]; [redacted], UK; Unpublished	No	-
Hunter, B., Graham, C., Prentice, D.E.	1975	A 3.9	Bronopol potential local and tumorigenic effects in repeated dermal application to mice (final report 0-80 weeks): [redacted]; [redacted], UK; Unpublished	No	-
Within US Environmental Protection Agency	1995	A 3.7.3.1 A 3.7.3.2 A 3.9 A 3.10.2	Reregistration Eligibility Decision, Bronopol, List B, Case 2770; 1995; Published	No	-
	2000a	A 3.8.1	Bronopol Bayer - Salmonella/microsome test – plate incorporation and preincubation method; [redacted], Bayer AG,	Yes	LANXESS Deutschland GmbH, Nutrition &

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			Wuppertal, Germany; report number [redacted] (study N° [redacted]); GLP; Unpublished		Biosciences (Switzerland) GmbH
	2000b	A 3.8.1	Bronopol Bayer - In vitro chromosome aberration test with Chinese Hamster V79 cells; [redacted], Bayer AG, Wuppertal, Germany; report number [redacted] (study N° [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2001a	A 3.8.1	Bronopol Bayer - V79/HRPT-test in vitro for the detection of induced forward mutations; [redacted], Bayer AG, Wuppertal, Germany; report number [redacted] (study N° [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2001b	A 3.8.2	Bronopol Bayer: Micronucleus-test on the male mouse; [redacted], Bayer AG, Wuppertal, Germany; report number [redacted] (study N° [redacted]); GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2001	A 3.8.2	Evaluation of Bronopol in the mouse bone marrow micronucleus test; [redacted], The Dow Chemical Company, Midland, Michigan, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2001	A 3.8.2	In vivo/in vitro unscheduled DNA synthesis in rat primary hepatocyte culture at two timepoints with a dose rangefinding assay with Bronopol; [redacted], Vienna Virginia, USA; Report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006	A 3.10.2	Bronopol: Oral gavage developmental toxicity study in New Zealand white rabbits; [redacted]; The Dow Chemical Company, Midland, Michigan, USA; report number: [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2007	A 3.10.2	Bronopol: Oral gavage developmental toxicity study in New Zealand white rabbits; [redacted]	Yes	Nutrition & Biosciences

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			[redacted]; The Dow Chemical Company, Midland, Michigan, USA; report number: [redacted]; GLP; Unpublished		(Switzerland) GmbH, LANXESS Deutschland GmbH
Irvine, L.	1992	not applicable	Bronopol: oral (gavage): rabbit developmental toxicity (teratogenicity) study; [redacted]; report (laboratory project) number: [redacted]; Unpublished	No	-
Palmer, K.	1995	not applicable	Bronopol: oral (gavage) rat developmental toxicity study; [redacted]; report (laboratory project) number: [redacted]; Unpublished	No	-
	2008	A 3.10.1	Bronopol: Two generation drinking water reproductive toxicity study in CRL:CD(SD) rats; [redacted]; The Dow Chemical Company, Midland, Michigan, USA; study number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006	A 4.2.3.1	Bronopol: A 96-hour flow-through acute toxicity test with the bluegill (Lepomis macrochirus); [redacted], Easton, Maryland, USA; [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2005	A 4.2.3.1	Bronopol: An acute toxicity study with the rainbow trout, Oncorhynchus mykiss; [redacted], The Dow Chemical Company, Midland, Michigan, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2012		2-Bromo-2-nitroethanol - Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions, Following OECD Guideline #203; [redacted], Wareham, Massachusetts, USA; Study no. [redacted] (report number [redacted]); GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2000	A 4.2.3.1	Bronopol Bayer: Acute Daphnia toxicity. In (pp. 19-27): Investigation of the ecological properties of Bronopol Bayer; Bayer AG, [redacted], Germany; Study no. [redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH

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	2006	A 4.2.3.1	Bronopol: A 96-hour flow-through acute toxicity test with the saltwater mysid (Americamysis bahia); [redacted], Easton, Maryland, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2012	A 4.2.3.1	2-Bromo-2-nitroethanol - Acute Toxicity to Water Fleas, Daphnia magna, Under Flow-Through Conditions, Following OECD Guideline #202; [redacted], Wareham, Massachusetts, USA; Report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006a	A 4.2.3.1	Bronopol: A 96-hour toxicity test with the freshwater alga (Scenedesmus subspicatus); [redacted], Easton, Maryland, USA; report number [redacted] GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006b	A 4.2.3.1	Bronopol: A 96-hour toxicity test with the freshwater alga (Anabaena flos-aquae); [redacted], Easton, Maryland, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2002	A 4.2.3.1	Bronopol: Growth inhibition test with the green alga, Selenastrum capricornutum; [redacted], Columbia, Missouri, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006c	A 4.2.3.1	Bronopol: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa); [redacted], Easton, Maryland, USA; report number [redacted] GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2006d	A 4.2.3.1	Bronopol: A 96-hour toxicity test with the marine diatom (Skeletonema costatum); [redacted], Easton, Maryland, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS

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					Deutschland GmbH
	2012	A 4.2.3.1	2-Bromo-2-nitroethanol - 72-Hour Acute Toxicity Test with Freshwater Green Alga, Pseudokirchneriella subcapitata, Following OECD Guideline 201; [redacted], Wareham, Massachusetts, USA; Study number [redacted] (report number [redacted]); GLP ; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2007	A 4.2.3.1	Bronopol : Sublethal toxic effects to rainbow trout (Oncorhynchus mykiss) in a fish juvenile growth test over 28 days; [redacted], Itingen, Switzerland; report number [redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH
	2004	A 4.2.3.1	Bronopol – Full life-cycle toxicity test with water fleas, Daphnia magna under flow-through conditions; [redacted], Wareham, Massachusetts, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH
	2006e	A 4.2.3.1	Bronopol: A 7-day static toxicity test with duckweed (Lemna gibba G3); [redacted], Easton, Maryland, USA; report number [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH
	1973	A 4.2.3.1	Rainbow Trout Toxicity (LC-50) Studies Tris Nitro; [redacted], Madison, Wisconsin, USA; Report No. [redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	1989	A 4.2.3.1	Acute Toxicity of Tris Nitro to Daphnia magna; [redacted], Aquatic Toxicology Division, Columbia, Missouri, USA; Report No. [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH
	2002	A 4.2.3.1	Tris(Hydroxymethyl) Nitromethane (TRIS NITRO™ SOLID CHT): Growth Inhibition Test With the Freswater Green Alga, Selenestra capricornutum PRINTZ; [redacted], The Dow Chemical Company, Midland, Michigan (USA); Report No. [redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH

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	1992	A 1.3	Explosibility Testing of 2-Bromo-2-nitropropane-1,3-diol (Bronopol, Myacide AS) for the Boots Company PLC, Nottingham; HSE Buxton, HSE No. [redacted], Boots Company PLC, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	2007a	A 1.3.1	Protectol BN - Other indications of flammability. Statement; BASF AG, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	2000	A 1.3	Crystalline Bronopol - Corrosion Potential. Experience in use; BASF MicroCheck Limited, UK; Unpublished	Yes	BASF SE
	1992	A 3.2.1	Bronopol: acute oral toxicity to rats; Boots Pharmaceuticals, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	2000	A 3.2.2	Myacide AS – Acute dermal toxicity study in rats; Experimental Toxicology and Ecology, BASF AG, Ludwigshafen/Rhein, Germany; [redacted]; GLP: Unpublished	Yes	BASF SE
	1986	A 3.2.3 A 3.4	Bronopol Boots: Acute inhalation toxicity study – rats: 4 hour exposure; [redacted], North Yorkshire, UK (sponsor: The Boots Company PLC., Nottingham, UK); [redacted] ([redacted]); Unpublished	Yes	BASF SE
	1987	A 3.3	Bronopol: EPA FIFRA/OECD acute dermal irritation/corrosion test in the rabbit; [redacted], Derby, UK (sponsor: Dynamit Nobel AG), BASF MicroCheck Ltd; [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1996	A 3.4	Primary eye irritation studies with bronopol in the rabbit. Knoll MicroCheck (D90); [redacted], Nottingham, UK; [redacted] (compilation of 3 studies, [redacted], [redacted] and [redacted], conducted between 1958 and 1972); Unpublished	Yes	BASF SE
	1993	A 3.1	[^{14}C] Bronopol: Excretion of radiolabelled material and terminal tissue distribution in tissues from male and female Charles River CD rats after administration of a single oral dose of [^{14}C] Bronopol (10 mg/kg); Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1993	A 3.1	[^{14}C] Bronopol: Excretion of radiolabelled material and terminal tissue distribution in tissues from male and female Charles River CD rats after administration of a single oral dose of [^{14}C] Bronopol (50 mg/kg); Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1993	A 3.1	Bronopol: Repeated oral administration: distribution and excretion in the rat; [redacted], Eye, Suffolk, England (sponsor: Boots Company plc, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE

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	1993	A 3.1	An investigation of the metabolites of bronopol in urine from male and female CD rats after administration of a single oral dose of [^{14}C] bronopol (10 or 50 mg/kg) or fourteen repeated daily doses of bronopol followed by a single dose of [^{14}C] bronopol (10 mg/kg); Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1974	A 3.1	The metabolism of bronopol (2-bromo-2-nitropropane-1,3-diol) after oral administration to rats and dogs, and application to the skins of rats and rabbits; [redacted], Huntingdon, UK (sponsor: The Boots Co. Ltd., Pharmaceutical Research, Nottingham, UK); [redacted]; Unpublished	Yes	BASF SE
	1993	A 3.1	An investigation of the metabolic fate of [^{14}C] bronopol in the skin following one or two topical applications of [^{14}C] bronopol to the shaved dorsal skin of male CFLP mice; The Boots Company plc, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1987	A 3.1	The absorption of [^{14}C] material following the repeated topical application of 0.5% w/v solution of [^{14}C] bronopol in 90% acetone: 10% water to a shaved area on the dorsum of male CFLP mice; The Boots Company plc, UK; [redacted]; Unpublished	Yes	BASF SE
	1993	A 3.1	Investigation of the metabolite, bromide ion, in male Charles River CD rats urine following single oral doses of bronopol (1 mg/kg and 50 mg/kg); Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1993	A 3.1	The measurement of bromide ion in rat urine using capillary electrophoresis; Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1973	A 3.7.1.2	Effect of repeated administration of bronopol to the skin of rabbit for three weeks; [redacted], Hundingdon, UK (sponsor: Boots Pure Drug Co. Ltd., UK); BTS42/73549 (E74002); Unpublished	Yes	BASF SE
	1973	A 3.7.2.1	Bronopol oral toxicity to rats, repeated administration for 13 weeks; [redacted], Hundingdon, UK (sponsor: Boots Pure Drug Co. Ltd., UK); [redacted], [redacted]; Unpublished	Yes	BASF SE
	1973	A 3.7.2.1	Boots: Bronopol oral toxicity study in the Beagle dog (initial study and repeated dosage for 13 weeks) repeated administration for 13 weeks; [redacted], Hundingdon, UK (sponsor: Boots Pure Drug Co. Ltd., UK); [redacted] & [redacted] ([redacted]); Unpublished	Yes	BASF SE

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	1986	A 3.8.1	Bronopol-Boots: In vitro bacterial mutagenicity testing; The Boots Company PLC Research Department, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1986	A 3.8.1	Bronopol-Boots: In vitro human lymphocyte clastogenicity testing; The Boots Company PLC Research Department, UK; T[redacted]; GLP; Unpublished	Yes	BASF SE
	1986	A 3.8.1	Bronopol-Boots: In vitro mammalian cell mutation assay; The Boots Company PLC Research Department, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1986	A 3.8.1	Bronopol-Boots: Micronucleus assay in mice; The Boots Company PLC Research Department, UK; [redacted]; GLP; Unpublished	Yes	BASF SE
	1998	A 3.8.2	Myacide AS (bronopol): Measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure; [redacted], Otley Road, Harrogate, North Yorkshire, UK (sponsor: Knoll MicroCheck, Nottingham UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1974	A 3.8.2	Bronopol: Mutagenicity testing by means of in vitro microbial tests, the host-mediated assay, and the dominant lethal assay in mice; The Boots Company Limited, Research Department, UK; [redacted]; Unpublished	Yes	BASF SE
	1986	A 3.8.1	Formaldehyde: in vitro human lymphocyte clastogenicity testing, Status: Breakdown product of bronopol; The Boots Company PLC Research Department; [redacted]; GLP; Unpublished	Yes	BASF SE
	1986	A 3.8.2	Bronopol Boots: Genotoxicity in short-term tests; The Boots Company PLC Research Department; [redacted]; GLP; Unpublished	Yes	BASF SE
	1976	A 3.7.3.1 A 3.9	Bronopol toxicity and tumorigenicity study in rats by administration in the drinking water for 104 weeks; [redacted], Huntingdon, Cambridgeshire, UK (Sponsor: Boots Pure Drug Co. Ltd); [redacted] ([redacted]); Unpublished	Yes	BASF SE
	1993	A 3.7.3.1 A 3.9	Bronopol toxicity and tumorigenicity study in rats by administration in the drinking water for 104 weeks: combined histopathology report; Pharmaceuticals Research Department, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	1985	A 3.7.3.1 A 3.9	Bronopol toxicity and tumorigenicity study in rats by administration in the drinking water for 104 weeks (Addendum to Report No: [redacted]); The Boots Company PLC Research Department; [redacted]; Unpublished	Yes	BASF SE
	1998	A 3.7.3.1	Bronopol: Tumorigenicity study in rats by administration in the drinking water - statistical analysis of mortality and forestomach papilloma tumour incidence; [redacted], Huntingdon, England (sponsor: [redacted]	Yes	BASF SE

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			[redacted], Nottingham, England); [redacted] ([redacted]); Unpublished		
	1986	A 3.7.3.1 A 3.9	Bronopol: doses given to rats in a two-year study: Statistical Report; [redacted]; Unpublished	Yes	BASF SE
	1975	A 3.7.3.2	Bronopol potential local and systemic tumorigenic effects in repeated dermal application to mice (Final report 0 – 80 weeks); [redacted], Huntingdon, Cambridgeshire, UK (Sponsor: The Boots Company Limited); BTS 43/74761 (E75004); Unpublished	Yes	BASF SE
	1986	A 3.7.3.2 A 3.9	Bronopol: potential local and systemic tumorigenic effects in repeated dermal application to mice. Histopathology supplement; The Boots Company PLC Research Department, UK; [redacted]; Unpublished	Yes	BASF SE
	1992	A 3.7.3.2 A 3.9	Bronopol - potential local and systemic tumorigenic effects in repeated dermal application to mice; Boots Pharmaceuticals Research Department, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	1998	A 3.7.3.2 A 3.9	Bronopol: Tumorigenic effects in repeated dermal application in mice - statistical analysis of mortality and skin papilloma tumour incidence, and nodular hyperplasia of the liver incidence; [redacted], Huntingdon, England (sponsor: [redacted], Nottingham, England); [redacted] ([redacted]); Unpublished	Yes	BASF SE
	1973	A 3.7.3.2 A 3.9	Preliminary tolerance test of bronopol in dermal application to mice; [redacted], Hundingdon, UK (sponsor: Boots Pure Drug Co. Ltd., UK); [redacted], ([redacted]); Unpublished	Yes	BASF SE
	1991	A 3.10.2	Bronopol Oral (gavage) rabbit developmental toxicity (teratogenicity) study; [redacted], Bromyard Road, Ledbury, Herefordshire, HR8 1LH, England (sponsor: Boots Pharmaceuticals, The Priory, Thurgarton, Nottingham, England); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1995	A 3.10.2	Bronopol Oral (gavage) rat developmental toxicity study; [redacted], Bromyard Road, Ledbury, Herefordshire, HR8 1LH, England (sponsor: Boots Pharmaceuticals, The Priory, Thurgarton, Nottingham, England); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1973	A 3.10.2	Effects of bronopol on peri- and post-natal development of the rat; [redacted], Huntingdon, UK (sponsor: Boots Pure Drug Company, Nottingham, UK); [redacted]; Unpublished	Yes	BASF SE
	1991	A 3.10.2	Bronopol Oral (gavage) rabbit teratology dose ranging study & Addendum; [redacted], Bromyard Road, Ledbury, Herefordshire, HR8 1LH, England (sponsor: Boots Pharmaceuticals, The Priory,	Yes	BASF SE

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			Thurgarton, Nottingham, England); [redacted] ([redacted]); GLP; Unpublished		
	1993	A 3.10.2	Bronopol Oral (gavage) rat developmental toxicity, dose-ranging study; [redacted], Bromyard Road, Ledbury, Herefordshire, HR8 1LH, England (sponsor: Boots Pharmaceuticals, The Priory, Thurgarton, Nottingham, England); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1987	A 3.10.1	Bronopol: Two-generation reproduction study in rats & Amendment (1991); [redacted], Mattawan, Michigan, USA (sponsor: The Boots Company PLC, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1973	A 3.10.1	Effect of bronopol on fertility and general reproductive performance of the rat; [redacted], Huntingdon, UK (sponsor: Boots Pure Drug Company, Nottingham, UK); [redacted] ([redacted]); Unpublished	Yes	BASF SE
	1986	A 3.10.1	Bronopol: Range-finding reproduction study in rats; [redacted], Mattawan, Michigan, USA (sponsor: The Boots Company PLC, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1973	A 3.16	Bronopol: Methaemoglobin formation in cats; The Boots Company PLC Research Department; [redacted]; Unpublished	Yes	BASF SE
Peters MS, Connolly SM, Schroeter AL	1983	A 3.3 A 3.5	Bronopol allergic contact dermatitis; Contact Dermatitis 9: 397-401; Published	No	Public
Storrs FJ, Bell DE	1983	A 3.5	Allergic contact dermatitis to 2-bromo-2-nitropropane-1,3-diol; Am Acad Dermatol 8: 157-170; Published	No	Public
Ford GP, Beck MH	1986	A 3.5	Reactions to Quaternium 15, Bronopol and Germall 115 in a standard series; Contact Dermatitis 14: 271-274; Published	No	Public
Frosch PJ, White IR, Rycroft RJG, Lahti A, Burrows D, Camarasa JG, Ducombs G, Wilkinson JD	1990	A 3.5	Contact allergy to Bronopol; Contact Dermatitis 22: 24-26; Published	No	Public
Frosch PJ, Weickel R	1987	A 3.5	Kontaktallergie auf das Konservierungsmittel Bronopol. (German Report); Hautarzt 38: 267-270; Published	No	Public

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Schnuch A, Geier J, Uter W, Frosch PJ,	1998	A 3.5	Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study; Br J Dermatology 138: 467-476; Published	No	Public
Shaw S	1997	A 3.5	Patch testing Bronopol; Cosmetics & Toiletries magazine 112: 67-73; Published	No	Public
	1984	A 3.1	An investigation of the absorption and excretion of [^{14}C] Bronopol in volunteers following the application to the skin of Soltan 3 cream containing 0.1% [^{14}C] Bronopol (preliminary report); The Boots Company, Research Department, Drug Metabolism and Medical Science, UK; [redacted]; Unpublished	Yes	BASF SE
	1984	A 3.1	An investigation of the absorption Bronopol in volunteers following the application to the skin of Soltan 3 cream containing 0.1% Bronopol; The Boots Company, Research Department, Drug Metabolism and Medical Science, UK; [redacted]; Unpublished	Yes	BASF SE
	1977	A 3.5	Bronopol Skin Sensitization Test (Study carried out by Professore HI Maibach, Dept of Dermatology, San Francisco Medical Centre, University of California, U.S.A); The Boots Company, Research Department, Product Registration (Pharmaceutical), UK; [redacted]; Unpublished	Yes	BASF SE
	1984	A7.4.1.1_02	Bronopol: acute toxicity to Bluegill Sunfish (Lepomis macrochirus); [redacted], Brixham, UK (sponsor: The Boots Company PLC, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1981	A 4.2.3.1	Determination of the acute toxicity of Myacide BT to Daphnia magna; [redacted], Brixham, UK (sponsor: The Boots Company LTD, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1998	A 4.2.3.1	The acute toxicity of bronopol to the marine copepod Acartia tonsa [redacted], Berkshire, UK (sponsor: Knoll MicroCheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1994	A 4.2.3.1	A study of the growth inhibition effects of Bronopol with 3 freshwater algal species to OECD 201; [redacted], Buckinghamshire, UK (sponsor: Boots Microcheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1998	A 4.2.3.1	The toxicity of bronopol to the freshwater unicellular green alga Scenedesmus subspicatus; [redacted], Berkshire, UK (sponsor: Knoll MicroCheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1998	A 4.2.3.1	The toxicity of bronopol to the marine diatom Skeletonema costatum; [redacted], Berkshire, UK (sponsor: Knoll MicroCheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE

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	2002	A 4.2.2	Determination of the inhibition of oxygen consumption by activated sludge in the activated sludge respiration inhibition test; BASF AG, Department of Experimental Toxicology and Ecology, Ludwigshafen/Rhein, Germany; [redacted]; GLP; Unpublished	Yes	BASF SE
	1996	A 4.2.2	An evaluation of the effect of Bronopol on the inhibition of activated sludge respiration (based on OECD guideline 209); [redacted], Bedfordshire, UK (sponsor: Knoll MicroCheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1996	A 4.2.3.1	The toxicity of Bronopol to the early life stages of rainbow trout (Oncorhynchus mykiss Walbaum); [redacted], Buckinghamshire, UK (sponsor: [redacted]); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1992	A 4.2.3.1	Bronopol: Reproduction test with Daphnia magna; [redacted], The Netherlands (sponsor: Boots Pharmaceuticals Ltd); [redacted]; GLP; Unpublished	Yes	BASF SE
	2012	A 4.2.3.1	2-Bromo-2-nitroethanol, Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions, Following OECD Guideline #203; [redacted] (Study Number: [redacted]); GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH
	2012	A 4.2.3.1	2-Bromo-2-nitroethanol - Acute Toxicity to Water Fleas, Daphnia magna, Under Flow Through Conditions, Following OECD Guideline #202; [redacted] (Study Number: [redacted]); GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH
	2012	A 4.2.3.1	2-Bromo-2-nitroethanol: 72-Hour Acute Toxicity Test with Freshwater Green Alga, Pseudokirchneriella subcapitata, Following OECD Guideline #201; [redacted] (Study Number: [redacted]); GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH
	1989	A 4.2.3.1	Acute Toxicity of Tris Nitro to *Daphnia magna*; [redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH
	2002	A 4.2.3.1	Tris(hydroxymethyl)nitromethane (TRIS NITRO™ SOLID CHT): Growth Inhibition Test With the Fresh Water Green Alga, *Selenastrum capricornotum* Printz; [redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
	1992	A 3.2.1	Bronopol: acute oral toxicity of technical material; Boots Pharmaceuticals, Nottingham, UK; [redacted]; Unpublished	Yes	BASF SE
	1976	A 3.5	Sensitization test on Bronopol; Environmental Safety Division, [redacted], Bedford, UK (sponsor: Boots); [redacted] ([redacted]); Unpublished	Yes	BASF SE
Anonymous	1984	A 3.3	Addendum to the final report on the safety assessment of 2-bromo-2-nitropropane-1,3 -diol. Cosmetic Ingredient Review; Journal of the American College of Toxicology, Vol. 3, No: 3: 139-155; Published	No	Public
	1984	A 4.2.3.1	Bronopol: Determination of acute toxicity to rainbow trout (Salmo gairdneri); [redacted], Brixham, UK (sponsor: The Boots Company PLC, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1984	A 4.2.3.1	Bronopol: Determination of acute toxicity to sheepshead minnow (Cyprinodon variegatus); [redacted], Brixham, UK (sponsor:The Boots Company PLC, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1994	A 4.2.3.1	A study of the growth inhibition effects of Bronopol with 3 freshwater algal species to OECD 201; [redacted], Buckinghamshire (sponsor: Boots Microcheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1998	A 4.2.3.1	The toxicity of bronopol to the freshwater unicellular green algae Scenedesmus subspicatus; [redacted], Berkshire, UK (sponsor: Knoll MicroCheck, Nottingham, UK); [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
	1987	A 3.2.1	Bronopol: OECD 401 acute oral toxicity test in the rat; [redacted], Derby, UK, (sponsor: Dynamit Nobel AG), BASF MicroCheck Ltd; [redacted] ([redacted]); GLP; Unpublished	Yes	BASF SE
Maibach H I	1977	A 3.3 A 3.5	Dermal sensitization potential of 2-bromo-2-nitropropan-1,3-diol (Bronopol ®); Contact Dermatitis 3, p 99; Published	No	Public
Rudner E J	1977	A 3.3	North American Group results; Contact Dermatitis 3: 208 - 209; Published	No	Public
Keldenich HP, Klein A	2011	A 1.3.1 B 1	Classification (CLP) for Preventol P-100 (Bronopol) CAS-No: 52-51-7; 2011/01604e; Published	No	LANXESS Deutschland GmbH
	2020	A 1.3.1	Protectol® BN; Determination of physico-chemical properties; Dust Explosibility in the Modified Hartmann-Apparatus (VDI 2263 Part 1); Lower explosion limit in the 20 L-sphere (DIN EN 14034-3); pmax and KSt-value in the 20 L-sphere (DIN EN 14034-1 / 14034-2); Minimum ignition energy of dust/air mixtures in the Mike3-Apparatus (DIN EN ISO 80079-20-2); Minimum ignition temperature of a dust cloud in the Godbert-Greenwald oven (DIN EN ISO 80079-20-2); Minumum ignition	Yes	BASF

Author(s)	Year	Section No / Reference No	Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published	Data Protection Claimed (Yes/No)	Owner
			temperature of a dust layer (5 mm) (DIN EN ISO 80079-20-2); study no. [redacted]; GLP; Unpublished		

Appendix VII: Study summaries (relevant for the CLH proposal)

DocIIIA attached as confidential documents.