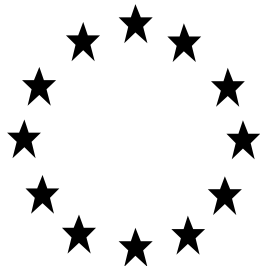
REGULATION (EU) NO 528/2012 CONCERNING THE MAKING AVAILABLE ON THE MARKET AND USE OF BIOCIDAL PRODUCTS

Assessment of active substances

RISK ASSESSMENT REPORT



2-bromo-2-nitro-1,3-propanediol

Product type 2, 11, 12

EC Number: **200-143-0**

CAS Number: **52-51-7**

Index Number: **603-085-00-8**

Applicant: **Bronopol Task Force, with its members LANXESS Deutschland GmbH, Microbial Control (Switzerland) GmbH and BASF SE**

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ASSESSMENT REPORT

SUMMARY

ECA PROPOSAL ON THE APPROVAL OF THE ACTIVE SUBSTANCE UNDER THE BPR

The overall conclusion of the eCA is that the bronopol in product type 2, 11 and 12 may be approved. The detailed grounds for the overall conclusion are described in the assessment report below.

1 PRESENTATION OF THE ACTIVE SUBSTANCE

1.1 IDENTITY OF THE ACTIVE SUBSTANCE

Table 1: Main constituent(s)

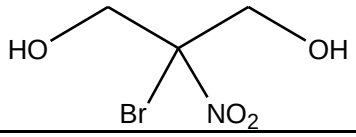
Main constituent	
ISO name	Bronopol
IUPAC or EC name	2-bromo-2-nitro-1,3-propanediol
EC number	200-143-0
CAS number	52-51-7
Index number in Annex VI of CLP	603-085-00-8
Minimum purity / content	≥98.90% w/w
Structural formula	C3H6BrNO4 

Table 2: Relevant impurities and additives

Relevant impurities and additives		
IUPAC name or chemical name or EC name	**Maximum concentration in % (w/w)**	**Index number in Annex VI of CLP**
Sodium bromide	≤0.1 % w/w dry weight	-

Impurities: The identity and content of the impurities is an industrial and commercial secret. Therefore, this information should be treated confidential. Please refer to the Confidential Section of the dossier.

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

1.2 INTENDED USES AND EFFECTIVENESS

Table 3: Use of the active substance

Product type	2
Intended use pattern	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 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 5: Use of the active substance

Product type	11
Intended use patterns	Preservatives for liquid-cooling and processing systems Bronopol is used as a cooling water preservative (*e.g.* in open and closed recirculating cooling systems). Preventive treatment with continuous dosing as well as curative treatment with shock dosing is intended. Within this assessment report, both preventive and curative use are 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 6: 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 7: Use of the active substance

Product type	12
Intended use patterns	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). Preventive treatment with continuous dosing as well as curative treatment with shock dosing is intended. Within this assessment report, both preventive and curative use are 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 8: 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 9: 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* Skin Irrit. 2 Eye Dam. 1 STOT SE 3 Aquatic Acute 1	H302 H312 H315 H318 H335 H400	GHS09 GHS05 GHS07 Dgr	H302 H312 H315 H318 H335 H400		Aquatic Acute M=10	
Dossier submitter's proposal	603-085-00-8	Bronopol; 2-bromo-2-nitropropane-1,3-diol	200-143-0	52-51-7	Modify: Acute Tox. 4* to Acute Tox. 3 Add: Acute Tox. 3 and Aquatic Chronic 1 Retain the rest	Modify: H302 to H301 Add: H331 and H410 Retain the rest	GHS09 GHS05 GHS06 Dgr	Modify: H302 to H301 And H400 to H410 Add: H331 Retain the rest	Add: EUH044	Modify: Aquatic Acute M=100 Add: ATE_{oral} = 193 mg/kg bw ATE_{dermal} = 1600 mg/kg bw $ATE_{inhalation}$ = 0.588 mg/L (dust/mist) Aquatic Chronic M=10	
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. 3 Acute Tox. 4 STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H331 H312 H335 H315 H318 H400 H410	GHS09 GHS05 GHS06 Dgr	H301 H331 H312 H335 H315 H318 H410	EUH044	ATE_{oral} = 193 mg/kg bw ATE_{dermal} = 1600 mg/kg bw $ATE_{inhalation}$ = 0.588 mg/L (dust/mist) Aquatic Acute M=100 Aquatic Chronic M=10	

* 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).

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 10: 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	Hazard class not applicable (e.g. physical state or chemical structure)	No
Corrosive to metals	Hazard class not applicable (e.g. physical state or chemical structure)	No
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	No
Acute toxicity via inhalation route	Harmonised classification proposed	Yes
Skin corrosion/irritation	Harmonised classification proposed	No
Serious eye damage/eye irritation	Harmonised classification proposed	No
Respiratory sensitisation	Data conclusive but not sufficient for classification	No
Skin sensitisation	Data conclusive but not sufficient for classification	No
Germ cell mutagenicity	Data conclusive but not sufficient for classification	No
Carcinogenicity	Data conclusive but not sufficient for classification	No
Reproductive toxicity	Data conclusive but not sufficient for classification	No

Specific target organ toxicity-single exposure	Harmonised classification proposed	No
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	No
Aspiration hazard	Hazard class not applicable (e.g. physical state or chemical structure)	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

Not relevant.

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

Table 11: Proposed Classification and Labelling according to Regulation (EC) No 1272/2008

Classification		Labelling				
Hazard Class and Category	Hazard statements	Pictograms	Signal word	Hazard statements	Suppl. Hazard statements	Precautionary statements
Acute Tox. 3 Acute Tox. 3 Acute Tox. 4 STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H331 H312 H335 H315 H318 H400 H410	GHS09 GHS05 GHS06	Danger	H301 H331 H312 H335 H315 H318 H410	EUH044	P261 P280 P264 P270 P271 P273 P280 P301+P310 P330 P302+P352 P304+P340 P305+P351+P338 P311 P321 P332+P313 P362+P364 P391 P403+P223 P405 P501

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 12: Packaging of the biocidal product

Type of packaging	Size/volume of the packaging	Material of the packaging	Type and material of closure(s)	Intended user (*e.g.* professional, non-professional)	Compatibility of the product with the proposed packaging materials (Yes/No)
Four panel bag ('big bag')	85x85x60 cm (LxBxH) 500 kg	PP 200g/m^2	Belt, PP 50 g/m	professional	Yes
Carton with inner liner	294x291x294 mm (LxBXH) 25 kg	corrugated cardboard, liner: PE plastic	Belt or tape	professional	Yes
Full open head telescoping fibre drum	25 kg, inner dimensions: 270mm diameter 415mm inside height Neck height: 65mm Outer dimensions: 280mm outside diameter 425mm outside height	plain kraft (brown) paper with PE inner liner (PE plastic, natural – no colour)	paper tape or a high quality plastic tape	professional	Yes

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

3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

3.1 SUMMARY OF THE ASSESSMENT OF EFFECTS ON HUMAN HEALTH

Table 13: Summary of the assessment of effects on human health

Endpoint	Brief description
Toxicokinetics	Based on the results of various studies, rapid and complete absorption (≥80% of applied dose) after oral exposure is concluded. It can also be concluded that dermal absorption of 43.6% is a scientifically justified, conservative approach for Bronopol in the presence of potential penetration enhancers (diluted product). Considering the irritant nature of Bronopol a dermal absorption of 43.6% can also be considered as a worst case assumption for the neat material/undiluted product. Moreover, in the absence of (substance-)specific information, complete absorption (100 %) is assumed for the inhalation route. Bronopol is widely distributed, and highest tissue residues (beside blood) were seen in fatty tissue, skin and organs involved in excretion, *i.e.* kidney, liver, and lung. 2-nitropropane-1,3-diol is the main metabolite in urine and plasma samples in rats and Bronopol was found to be rapidly excreted, mainly *via* urine (67-83%) whereas excretion *via* the faeces and exhaled air accounted for less than 10% each and played rather a minor role. There is no relevant accumulation potential for Bronopol.
Acute toxicity	Based on the results of the acute toxicity studies available, an acute toxicity potential can be concluded for Bronopol. Bronopol has a harmonised classification as Acute tox Cat. 4 H302 and Acute tox Cat. 4 H312, but based on the results of the oral toxicity studies, Acute tox Cat. 3 (H301) is proposed. In addition, bronopol needs to be classified as Acute tox Cat. 3 (H331) under Regulation (EC) No 1272/2008.
Corrosion and irritation	Bronopol causes skin irritation and eye damage. Bronopol is harmonised classified as Skin irritant Cat. 2 H315 and Eye damage Cat. 1 H318.
Sensitisation	Bronopol is not skin sensitising. Bronopol was found negative in two Guinea pig maximisation tests (OECD TG 406) and an LLNA test (OECD TG 429). In healthy volunteers, Bronopol did not induce skin sensitisation, while low incidences of dermal allergic responses were found in contact dermatitis patients.
Repeated dose toxicity	Short-term exposure to Bronopol mainly caused local effects at the application site (skin and stomach) in rabbits and dogs, respectively. Sub-chronic exposure to Bronopol mainly affected water and food consumption, body weight as well as kidney weights (including signs of nephropathy) in rats. The main effects following long-term (chronic) exposure to Bronopol included reduced body weight development and food/water consumption as well as changes in the kidney (increased weight, histopathological findings) and local irritation in the gastrointestinal tract in rats.
Genotoxicity	It cannot be concluded that Bronopol does not induce genotoxicity *in vivo*. 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. Also, mutagenicity studies in mammalian cells are of very low quality and do not allow a clear result to be obtained. 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. Results from three *in vivo* test systems give no indication that Bronopol causes genotoxic effects *in vivo*, both in somatic and germ cells. However, UDS assays are known to be not sufficiently reliable due to their lack of sensitivity (only large repair areas can be detected).
Carcinogenicity	Carcinogenicity of Bronopol was assessed after oral and dermal exposure to rats and mice, respectively. Incidental findings were reported in both studies, which occurred in both treated and untreated animals, and showed no dose-response relationship. With no treatment-related significant increase in neoplastic findings after chronic oral and dermal exposure of rats and mice, Bronopol is considered not carcinogenic.
Reproductive toxicity	Toxicity to reproduction was assessed in two 2-generation studies and a 1-generation study in the rat. Overt signs of systemic toxicity at the highest dose level were decreased body weight gain and food consumption, altered absolute and relative liver and kidney weights. No effects on the reproduction at dose levels lacking clear systemic toxicity were observed. The key values are based on the most reliable, most conservative 2-generation study available for Bronopol. Developmental toxicity of Bronopol was assessed in the rat and in rabbits. Pups from rabbits treated with a Bronopol dose causing maternal toxicity (including decreased body weight gain and food consumption) showed some effects clearly linked to maternal toxicity (reduced foetal body weight and incidental abnormalities of general retardation of skeletal ossification and growth), whereas rat pups were unaffected up to the maximum tolerable dose level. The key values are based on the most reliable, most conservative developmental toxicity study available for rabbits exposed to Bronopol.
Neurotoxicity	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. 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.
Immunotoxicity	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. 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.
Disruption of the endocrine system	Bronopol has been extensively studied, with a database containing information for all levels described in the OECD CF 2012 and in the ECHA/EFSA Guidance on the identification of endocrine disruptors. Level 1 is covered by *in silico* tools implemented in the Danish QSAR Database, the Endocrine Disruptome and the Estrogen

	Receptor binding model implemented in the OECD QSAR Toolbox. These have been assessed to evaluate the computational prediction of the potential of bronopol to interact with relevant receptors. Overall, these tools consistently provide no evidence of any endocrine activity of Bronopol. Level 2 is covered for EAS-modalities. Bronopol has been assessed in a series of *in vitro* assays, including investigations of estrogen and androgen receptor binding, estrogen, androgen, and thyroid receptor transactivation, and steroidogenesis. Information was derived from the ToxCast and EDSP21 databases as well as publicly available literature. Overall, these *in vitro* assays do not indicate any endocrine activity of Bronopol, as supported by the negative predictions of the level 1 models. Level 3 contains no information due to the absence of adverse effects in levels 4 and 5 and of activity in levels 1 and 2 and the lack of need to establish a MoA. Level 4 studies are *in vivo* repeated dose toxicity studies, including sub-acute, sub-chronic, chronic and developmental toxicity studies , providing information on adverse effects on endocrine relevant endpoints. Changes in organ weights and histopathology were generally secondary to systemic toxicity, *e.g.* reduced body weight, and were without histologic correlate. Evidence of target organ toxicity found in kidney due to significant histopathological changes and impact on kidney weight in rats in several studies Further, no pattern of effects was observed across available studies. No further treatment-related changes were observed in any organ in the available studies. Overall, these studies do not provide consistent evidence for an endocrine disruptive potential of Bronopol. Level 5 studies include multi-generation toxicity studies. The available 2-generation reproductive toxicity studies showed no treatment-related effect of bronopol in absence of marked systemic toxicity. The information derived from these studies were evaluated together with available level 4 studies in a weight of evidence approach. To conclude, the EATS-mediated parameters have been sufficiently investigated with regard to humans. The available mammalian toxicity studies demonstrate that the principal target organ of bronopol is the kidney, wherein adversity is not considered to be mediated by an endocrine mode of action. No pattern of bronopol-related adverse effects in endocrine-sensitive organs or endpoints was identified in available OECD CF levels 4 and 5 *in vivo* toxicity studies. No causal or mechanistic link could be established between bronopol and EATS-specific effects, and the available OECD CF levels 1 and 2 *in silico* and *in vitro* data did not provide evidence of an endocrine MoA. Conclusively, as the available animal studies do not provide consistent evidence for any EATS-related adversity which may be linked to an endocrine activity, the substance does not meet the ED criteria with regard to humans. It is possible conclude that bronopol is sufficiently investigated, and no adversity based on "EATS-mediated" parameters were found, thus the general conclusion is that bronopol does not met ED criteria as there is no "EATS-mediated" adversity. This is the Scenario 1a included in the Table 5 of ECHA/EFSA Guidance.
Other effects	No other adverse health effects were reported.

3.2 REFERENCE VALUES

Table 14: Reference values

	Study	NOAEL/ LOAEL	Overall assessment factor	Value
AEL	90-day oral toxicity study in dogs (A6.4.1_02)	8 mg/kg bw/day ($NOAEL_{oral}$)	100	0.08 mg/kg bw/day
ADI	90-day oral toxicity study in dogs (A6.4.1_02)	8 mg/kg bw/day ($NOAEL_{oral}$)	100	0.08 mg/kg bw/day
ARfD	90-day oral toxicity study in dogs (A6.4.1_02; based on vomiting few minutes after each dose)	8 mg/kg bw/day ($NOAEL_{oral}$)	100	0.08 mg/kg bw
AEC_{inhal}short-term	Acute inhalation toxicity study (A6.1.3_01)	89 mg/m³ ($NOAEC_{inhal}$)	25	3.6 mg/m³
$NOAEC_{irrit}$	Patch test in humans (A6_01_5-4; A6.12.6_03)	0.5%	-	-

3.3 RISK CHARACTERISATION

Table 15: Summary of exposure scenarios for PT02[1]

Summary table: scenarios			
Scenario number	**Scenario**	**Primary or secondary exposure Description of scenario**	**Exposed group**
2	Application	Primary exposure - Application of final end-use product to the waste holding tank and emptying of collecting tanks after usage	Professionals
3	Application	Primary exposure - Emptying of collecting tanks after usage	Professionals
4	Application	Primary exposure - Application of the final end-use product to chemical toilet. Manual application	Non-professionals
5	Application	Primary exposure - Emptying of the chemical toilet waste tank	Non-professionals
6	Post-application	Secondary exposure - General public in contact to spillage during the use of chemical toilets	General public
7	Post-application	Secondary exposure - Inhalation of volatilized residues	General public
7a	Post-application	Secondary exposure - Exposure to vapour to formaldehyde	General public

[1] This table is a copy of the table in Chapter 8.3 of the Assessment Report.

Table 16: Summary of exposure scenarios for PT11[2]

Summary table: scenarios			
Scenario number	**Scenario**	**Primary or secondary exposure Description of scenario**	**Exposed group**
2.	Application	**Primary exposure.** Biocidal product dilution transferred to the sump.	Professionals
3.	Post-application exposure	**Primary exposure.** Exposure towards residues during cleaning of the dispensing pumps.	Professionals
4.	Post-application exposure	**Primary exposure.** Exposure towards residues during system inspection and monitoring	Professionals
5.	Post-application exposure	**Primary exposure.** Exposure towards residues during cleaning of the fouled systems	Professionals
6.	Post-application exposure	**Primary exposure.** Exposure during disposal of waste	Professionals
7.	Post-application exposure	**Secondary exposure.** Secondary exposure - Exposure to aerosols	Bystanders

Table 17: Summary of exposure scenarios for PT12[3]

Summary table: scenarios			
Scenario number	**Scenario**	**Primary or secondary exposure Description of scenario**	**Exposed group**
1.	Mixing/Loading	**Primary exposure:** Solid (powder) loading/dumping	Industrials
2.	Post-application: cleaning	**Primary exposure:** Post-application - cleaning of the dispensing pumps	Professionals
3.	Post-application: water sampling	**Primary exposure:** Post application - process water sampling	Professionals
4.	Post-application: maintenance	**Primary exposure:** Post application - process equipment maintenance and RO systems	Professionals
5.	Post-application: disposal of waste	**Primary exposure:** Post-application - disposal of waste	Professionals
6.	Post-application from papermills	**Secondary exposure** from papermills: vapour phase [6a], aerosol phase [6b and 6c]	Professionals
7.	Post-application from papermills	**Secondary exposure** from papermills: contact with paper	Professionals

[2] This table is a copy of the table in Chapter 8.3 of the Assessment Report.
[3] This table is a copy of the table in Chapter 8.3 of the Assessment Report.

Table 18: Conclusion of risk characterisation for PT02

Summary table: human health scenarios			
Scenario	**Primary or secondary exposure and description of scenario**	**Exposed group**	**Conclusion**
Application	**Primary exposure** Application of biocidal product to the waste holding tank and emptying of collecting tanks after usage PPE: protective gloves RMM for medium and low hazard class chemicals (chemical googles, face shield, substance/task appropriate gloves/respirator, protection coverall)	Professionals	Acceptable with PPE and RMMs.
Application	**Primary exposure** Emptying of collecting tanks after usage	Professionals	Acceptable
Application	**Primary exposure** Application of the biocidal product concentrate to chemical toilet RMM for medium and low hazard class chemicals (Labelling, instructions for use, Childproof closure, Packaging eliminating exposure)	Non-Professionals	Acceptable with RMMs.
Application	**Primary exposure** Emptying of the chemical toilet waste tank	Non-Professionals	Acceptable
Post-Application	**Secondary exposure** Contact to spillage during the use of chemical toilets	General Public	Acceptable
Post-Application	**Secondary exposure** Inhalation of volatilized residues	General Public	Acceptable
Post-Application	**Secondary exposure** Exposure to vapour to formaldehyde	General Public	Acceptable

Table 19: Conclusion of risk characterisation for PT11

Summary table: human health scenarios			
Scenario	**Primary or secondary exposure and description of scenario**	**Exposed group**	**Conclusion**
Post-application	**Primary exposure** Exposure towards residues during cleaning of the dispensing pumps.	Professionals	Acceptable
Post-application	**Primary exposure** Exposure towards residues during system inspection and monitoring	Professionals	Acceptable
Post-application	**Primary exposure** Exposure towards residues during cleaning of the fouled systems	Professionals	Acceptable
Post-application	**Primary exposure** Exposure during disposal of waste	Professionals	Acceptable
Post-Application	**Secondary exposure** Exposure to aerosols	General Public	Acceptable

Table 20: Conclusion of risk characterisation for PT12

Summary table: human health scenarios			
Scenario	**Primary or secondary exposure and description of scenario**	**Exposed group**	**Conclusion**
Post-application	**Primary exposure** Cleaning dispensing pumps PPE: protective gloves RMM for medium and low hazard class chemicals (chemical googles, face shield, substance/task appropriate gloves/respirator, protection coverall)	Professionals	Acceptable with PPE and RMMs
Post-application	**Primary exposure** Process water sampling	Professionals	Acceptable
Post-application	**Primary exposure** Process equipment maintenance	Professionals	Acceptable
Post-application	**Primary exposure:** Disposal of waste	Professionals	Acceptable
Post-application	**Secondary exposure** Post-application from papermills (vapour phase)	Professionals	Acceptable
Post-application	**Secondary exposure** Post-application from papermills (aerosol phase)	Professionals	Acceptable
Post-application	**Secondary exposure** Secondary exposure to workers from papermills (contact with paper)	Professionals	Acceptable

4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

4.1 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Bronopol rapidly degrades in natural waters by abiotic degradation as well as biotically, but it does not fulfil readily biodegradability conditions. Further, some transformation products have been detected in the hydrolysis studies (abiotically mainly 2-bromo-2-nitroethanol (2-BNE) as transient product via reversible reactions) and in the STP simulation study (biotic process, mainly 2-Hydroxymethyl-2-nitro-1,3-propanediol (TNM)).

Bronopol hydrolyses rapidly at neutral and basic pH, leading to several routes of hydrolysis. The analytical identification during hydrolysis studies has led to concentrations of some identified and unidentified metabolites higher than 10%. Same occurs in the STP simulation test, where the parent disappears quite rapidly, and other metabolites are being formed, such as TNM.

Regarding bromide ion release, even though this has not been detected in the studies, this ion might be liberated in the STP when bronopol or 2-BNE reacts to other transformation products such as nitromethane. According to 2,2-dibromo-2-cyanoacetamide (DBNPA) opinion (December 2021), the bromide ion is causing the ED properties of the a.s. (thyroid). This is not the case for Bronopol, which has not shown effects on the thyroid according to the available studies. Nevertheless, calculation of bromide contribution to background levels should be included in the CAR as it was done for DBNPA. The concentration in the STP effluente for each PT scenario will be used to get the stechiometric bromide released from Bronopol to be compared to background levels. The calculations are included in appendix III.

Regarding the exposure assessment, all intended uses are going to emit through the STP, hence the receiving compartments are: STP, indirect releases to surface water, sediment, soil (via sludge) and groundwater. The fraction released to air is negligible due to bronopol properties.

Table 21: Summary table on compartments exposed and assessed

Compartment	Exposed (Y/N)	Assessed (Y/N)
STP	Y	Y
Freshwater	(Y)	(Y)
Sediment	(Y)	(Y)
Soil	(Y)	(Y)

Table 22: Summary table on relevant metabolites/degradants

Metabolite/ degradant/transformation- or reaction product	Compartment	% Active Substance
2-bromo-2-nitroethanol (2-BNE)	Freshwater	n.d.
2-Hydroxymethyl-2-nitro-1,3-propanediol (trade name: Tris(hydroxymethyl)nitromethane) (TNM)	STP	n.d.
Bromonitromethane (BNM)	Freshwater	n.d.
2-bromoethanol (2BE)	Freshwater	n.d.
Nitromethane	Freshwater	n.d.

n.d. = not determined

Table 23: Summary table on relevant physico-chemical and fate and behaviour parameter of the active substance

	Value	Unit	Remarks
Molecular weight	199.99	g/mol	
Log Octanol/water partition coefficient (Log Kow)	log Pow: -0.32 at 10 °C -0.42 at 20 °C -0.50 at 30 °C	Log 10	All at pH 3-4
Organic carbon/water partition coefficient (Koc)	136	L/kg	

Henry's Law Constant (25 °C)	$1.16*10^{-6}$	$Pa/m^3/mol$	
Biodegradability	Not readily biodegradable (QSAR prediction: YES)		Bronopol primarily degrades fast but inherent degradability test is only considered as supporting information and readily biodegradation is not fulfilled
DT50 for biodegradation in surface water	n/a	d or hr (at 12 ºC)	
DT50 for hydrolysis in surface water	0.36	d (at 12 ºC / pH 7)	The study indicated a DT50 of 35.28 minutes at pH 7 and 50 ºC (CAKE)
DT50 for photolysis in surface water	20-21	d	
DT50 for degradation in soil	300	d (at 12 ºC)	Default value based on BPR guidance vol IV, part B+C
DT50 for degradation in air	n/a	d or hr	
DT50 for degradation in sediment	n/a	d or hr	
Bioconcentration, aquatic	n/a		
Bioaccumulation, aquatic	n/a		(BCFWIN v2.17): Log BCF = 0.500 (BCF = 3.162)
Bioconcentration, terrestrial	n/a		
Bioaccumulation, terrestrial	n/a		

Table 24: Summary table on relevant physico-chemical and fate and behaviour parameter of the degradation products

Degradation products	TNM	2-BNE
Molecular weight	151.12	169.96
Log Octanol/water partition coefficient (Log Kow)	Log Kow (KOWWIN v1.68 estimate) = -1.66	Log Kow (KOWWIN v1.67 estimate) = -0.74
Organic carbon/water partition coefficient (Koc)	10 L/kg (MCI method)	1.458 L/kg (PCKOCWIN v1.66)
Henry's Law Constant (25 °C)	[HENRYWIN v3.20]: 4.88E-007 $Pa-m^3/mole$	2.36E-010 $atm-m^3/mole$ (estimated by Bond SAR Method)
Biodegradability	Not readily biodegradable (QSAR prediction: YES)	Not readily biodegradable
DT50 for biodegradation in surface water	n/a	n/a
DT50 for hydrolysis in surface water	3.42 d at 25 ºC	7 days at 20 ºC or 13 days at 12 ºC
DT50 for photolysis in surface water	n.a.	n.a.
DT50 for degradation in soil	n.a.	n.a.
DT50 for degradation in air	n/a	n.a.
DT50 for degradation in sediment	n/a	n.a.
Bioconcentration, aquatic	n/a	n.a.
Bioaccumulation, aquatic	3.162 L/kg wet-wt (BCFBAF v3.01)	(BCFWIN v2.17): Log BCF from regression-based method = 0.500 (BCF = 3.162)
Bioconcentration, terrestrial	n/a	n/a
Bioaccumulation, terrestrial	n/a	n/a

4.2 EFFECTS ASSESSMENT

Table 25: Summary table on calculated PNEC values for bronopol

Compartment	PNEC
Freshwater	0.00048 mg a.s./L
Sediment	0.0083 mg a.s./kg dw
STP	0.43 mg a.s./L (based on nominal)
Soil	0.21 mg a.s./kg d.w. (based on nominal)

Metabolite TNM is mainly formed in the STP simulation test (OECD TG 314B study), reaching a 92.5% at 2 h and decreasing below 5% after 72 h.

Hence, TNM should be assessed together with the parent. The CAKE simulation revealed a 99.75% of maximum TNM formation from Bronopol (as described in TAB ENV 213, the molar fraction of the parent molecule which is transformed into the specific TP should be summed over the whole time period of parent substance degradation, which results in this 99.75%):

Parameter	Initial Value	Bounds	Fixed
BN_0	90.96	0 to (unbounded)	No
k_BN	181.8	0 to (unbounded)	No
f_BN_to_TNM	0.9975	0 to 1	No
TNM_0	0	0 to (unbounded)	Yes
k_TNM	0.5666	0 to (unbounded)	No

Decay Times:

Compartment	DT50 (days)	DT90 (days)
BN	0.00452	0.015
TNM	1.17	3.9

SFO kinetics gives a DT50 = 1.17 days at study temperature (20.55 ºC) and an equivalent degradation rate of 0.01474 h-1 at 15 ºC of TNM in the STP, which results in a 14.46% degradation in the STP (estimated by simple treat). The PNEC values for the aquatic compartment are derived from acute ecotoxicity tests (available for algae, daphnia and fish) with the degradation product.

Table 26: Summary table on calculated PNEC values for TNM

Compartment	PNEC for TNM
Freshwater	0.0045 mg/L
Sediment	0.0207 mg/kg dw
STP	n/a
Soil	Not applicable as 0% TNM will partition to sludge (Simple treat for TNM*)

*Summary of simulated distribution in the STP (simple treat 4.0) for TNM: 0,0% to air, 85.54% to water, 14.46% degraded and 0% sludge. No sorption to sludge is expected for TNM; this concerns sorption to secondary sludge as the metabolite is formed in the aeration tank. Primary sludge is not relevant for the metabolite.

Regarding hydrolysis products such as 2-BNE, they may be present in cooling fluids and paper pulp due to higher temperatures which enhances hydrolysis. However, taking into account the similar ecotoxicological profile, it has been considered that the risks have been sufficiently covered by the assessment for the parent compound. The hydrolysis is taking place in any process before the STP, but as there is no direct emission to surface water, it will always go through the STP, and the degradation in the STP is very high for 2-BNE (DT50 = 0.0047 h), hence, it is very unlikely that 2-BNE reach surface water. The WGIV2022 decided that 2-BNE is covered by Bronopol only when emission is through STP, where 2-BNE is very rapidly degraded. In case of any direct emission for a product authorisation, 2-BNE should be assessed.

4.3 EXPOSURE ASSESSMENT

Table 27: Summary table on calculated PEC values for Bronopol

	PEC_{STP} [mg/L]	**PECwater** [mg/L]	**PECsed** [mg/kgdwt]	**PECsoil** (mg/kgwwt)	**PEC_{GW}*** [µg/L]	**PECair** [mg/ m^3]
PT 2						
Scenario 1: Chemical toilets	1.03E-03	1.03E-04	1.77E-03	2.39E-03	0.77	n.a.
PT 11						
Scenario 2: small open recirculating cooling systems – Continuous dosing	1.97E-04	1.97E-05	3.39E-04	4.58E-04	0.15	n.a
Scenario 3: small open recirculating cooling systems – Shock dosing	8.54E-04	8.54E-05	1.47E-03	1.99E-03	0.64	n.a
Scenario 4: Closed recirculating cooling systems – Continuous dosing a)Drainage (worst case) b)With degradation	a)2.10E-03 b)1.81E-07	a)2.10E-04 b)1.81E-08	a)3.62E-03 b)3.11E-07	a)4.89E-03 b)4.21E-07	a)1.58 b)1.36E-04	n.a
PT 12						
Scenario 5: Slimicide Paper Industry Typical case	2.17E-02	1.08E-04	1.86E-03	5.99E-02	19.4	n.a
Scenario 6: worst case STP	1.06E-01	5.32E-04	9.14E-03	2.93E-01	94.9	n.a.
Scenario 7: worst case direct release DF 1000**	n.a.	1.13E-03	1.95E-02	n.a.	n.a.	n.a.
Scenario 8: small factories typical	2.17E-02	2.17E-03	3.73E-02	2.04E-02	6.59	n.a.
Scenario 9: small factories worst	1.06E-01	1.06E-02	1.83E-01	2.71E-02	8.77	n.a.

*Tier 1. PECGW will be refined by using a simulation tool (FOCUS pearl 4.4.4.)

**Worst case very unlikely due to BAT document including secondary treatment for paper mills > 20 ton/day

Table 28: Summary table on calculated PEC values for TNM

TNM (no partition to sludge, hence no risk to soil or GW applicable*)	**PECwater** [mg/L]	**PECsed** [mg/kgdwt]
PT2 – Scenario 1: Chemical toilets	2.27E-03	1.04E-02
PT11		
Scenario 2: Open recirculating cooling systems – Continuous dosing	4.35E-04	2.00E-03
Scenario 3: Open recirculating cooling systems – Shock dosing	1.88E-03	8.67E-03
Scenario 4: Closed recirculating cooling systems – Continuous dosing a)Drainage (worst case) b)With degradation	a) 4.64E-03 b) 3.99E-07	a) 2.13E-02 b) 1.84E-06
PT12		
Scenario 5: Slimicide Paper Industry typical case scenario	2.40E-03	1.10E-02
Scenario 6: worst case with STP	1.17E-02	5.40E-02
Scenario 8: Small factories typical case	4.79E-02	2.20E-01

*The degradation product TNM is formed in the STP but it does not partition to sludge to soil. Nevertheless, a worst case has been considered: all bronopol released to the soil compartment from sludge application, once in soil, degrades to TNM at a rate of 100%. By considering the OECD TG 307 pretest results and FOCUS refinement when needed, there is no risk to groundwater from TNM.

4.4 RISK CHARACTERISATION

Table 29: Summary table on calculated PEC/PNEC values for Bronopol

	PEC/PNEC STP	PEC/PNEC water	PEC/PNEC sed	PEC/PNECsoil	PEC_{GW} (trigger value of 0.1 ug/l)	PEC/ PNEC air
PT 2						
Scenario 1: Chemical toilets	2.39E-03	2.14E-01	2.14E-01	1.29E-02	**0.77**	n.a.
PT 11						
Scenario 2: Open recirculating cooling systems – Continuous dosing - small	4.58E-04	4.11E-02	4.09E-02	2.48E-03 Direct release: 8.42E-03 (no drift elim.) 8.42E-05 (99% elim)	**0.15** Direct release: 0.62 (no drift elim.) 0.03 (99% elim)	n.a
Scenario 3: Open recirculating cooling systems – Shock dosing - small	1.99E-03	1.78E-01	1.77E-01	1.07E-02 Direct release: 3.65E-02 (no drift elim.) 3.65E-04 (99% elim)	**0.64** Direct release: 2.68 (no drift elim.) 0.03 (99% elim)	n.a
Scenario 4: Closed recirculating cooling systems – Continuous dosing. a)Drainage (worst case) b)With degradation	a)4.89E-03 b)4.21E-07	a)0.44 b)3.77E-05	a)0.44 b)3.76E-05	a)2.64E-02 b)2.28E-06	a)1.58 b)1.36E-04	n.a
PT 12: slimicide, paper industry						
Scenario 5: typical case	5.04E-02	2.26E-01	2.25E-01	3.24E-01	**19.4**	n.a
Scenario 6 worst case with STP	2.47E-01	**1.11**	**1.10**	**1.59**	**94.92**	n.a
Scenario 7 worst case DF 1000	n.a	**2.36**	**2.35**	n.a	n.a	n.a
Scenario 8 small factories typical case	5.05E-02	**4.52**	**4.51**	1.10E-01	**6.59**	n.a
Scenario 9 small worst case	2.47E-01	**22.15**	**22.09**	1.46E-01	**8.77**	n.a

Table 30: Summary table on calculated PEC/PNEC values for TNM

Summary table on calculated PEC/PNEC values for TNM		
	PEC/PNECwater	**PEC/PNECsed**
PT 2, Scenario 1: Chemical toilets	0.5	0.5
PT 11		
Scenario 2: small open recirculating cooling systems – Continuous dosing	0.10	0.10
Scenario 3: small open recirculating cooling systems – Shock dosing	0.42	0.42
Scenario 4: Closed recirculating cooling systems – Continuous dosing. a)Drainage (worst case) b)With degradation	a) **1.03** b) 8.88E-05	a**) 1.03** b) 8.88E-05
PT12		
Scenario 5: Slimicide Use in Paper industry, typical case scenario	0.53	0.53
Scenario 6: Worst case with STP	**2.61**	**2.61**
Scenario 8: Small paper factories typical case	**10.65**	**10.65**

Conclusion:

All calculated PEC/PNEC ratios for sewage treatment plant (STP) are far below the trigger value of RCR = 1, confirming acceptable risk for this environmental compartment considering the intended biocidal applications.

For the intended applications, calculated PEC/PNEC values for surface water and sediment are below the trigger value of RCR = 1, confirming acceptable risk for these environmental compartments, except for:

- The realistic worst-case in PT12, direct release to surface water, which is not allowed according to BPC-48 "Application is only allowed in paper factories that comply with the Industrial Emission Directive 2010/75/EU where wastewater is purified in an on-site industrial sewage treatment plant including a biological treatment step in accordance to the Best Available Techniques (BAT) as prescribed in the BAT-reference document (BREF) for the production of pulp, paper and board".
- The worst case with STP, with a slight excedance of the PEC/PNEC ratio.
- Small paper factories, exempted from 2010/75/EU (production capacity not exceeding 20 tonnes per day) with a discharge to a municipal STP. A safe use should be demonstrated at product level based on monitoring data or other ways for a refinement.
- The closed recirculating cooling systems during drainage, if no degradation is considered (worst case), due to TNM, with a slight excedance.

Exposure to seawater/-sediment is not expected, hence a risk assessment for these compartments is not required.

Negligible emissions to air are expected due to the a.s. properties.

Regarding soil, there is acceptable risk for the use of Bronopol. In the direct release from open cooling systems, a drif elimination is required to obtain a safe use.

Although not likely, application to soil of a sludge from an industrial STP in a paper factory cannot be excluded in this specific case. Hence, risk assessment to soil and groundwater has been assessed as well, leading to risk to groundwater in all cases and a slight exceedance of the RCR for soil in the worst case scenario with STP.

The risk in groundwater (>0.1 µg/L) must be refined by FOCUS pearl 4.4.4., giving unacceptable risk for the following uses after tier 2 with FOCUS:

PT2 (worst case value 0.43)

PT11: open shock system (worst case value 0.36)

PT11: close drainage without degradation (worst case value 0.88)

PT12: small factories (releasing to municipal STP, sludge could be applied to soil) (worst case value 3.64)

A weight of evidence approach was agreed at ENV WGIII2023 AHF. This WoE would be enough to demonstrate a safe use, based on:

- Rapid Hydrolysis of Bronopol at ambient T and pH.
- Rapid degradation of Bronopol in all acute ecotoxicity studies.
- Rapid degradation in the ready biodegradability tests (borderline case).
- No bronopol has been detected (below LOD) in municipal STP sludge (monitoring data), applicable for PT2 and PT11. These monitoring data published from several sources (please see section A.4.1.4) showed that no Bronopol had been detected in any environmental compartment (below LOD) including municipal sludge and even industrial STP sludge from paper factories. For instance, the LOD in the Swedish monitoring study for sludge was 12 - 24 µg/kg dwt. Bronopol could not be detected in sludge and therefore its concentration was in all cases < LOD. Still, taking the upper LOD with 24 µg/kg dwt as input parameter for Csludge, the resulting application concentrations are: Arable: 1.20E-4 kg/ha and Grass: 2.40 E-5 kg/ha. This leads to groundwater concentrations according to FOCUS Pearl 4.4.4 of 0.006 µg/L for arable and 0.001 µg/L for grass. As a result, bronopol would not pose any risk to the groundwater compartment.
- Risk to groundwater is due to the actual modelling, but very unlikely as only 1% partitions to sludge. The degradation in the primary settler will be quite important and almost no Bronopol is expected into the primary sludge.
- For PT2, and only to be used as supporting information, there is a test in urine showing rapid degradation of Bronopol in such media.
- There is an on-going OECD TG 307 study which has been required to the applicants. The pre-test OECD TG 307 results gives a worst case DT50 of around 5 days in soil at 20 ºC (9.3 days at 12 ºC). This value cannot be used for further risk assessment at product level. This is being used as supporting data at active substance level.
- Degradation of around 10% of Bronopol via hydrolysis will take place in the sewer system following equation 1 at 12 ºC and with a HRT of 1 h: Csew,eff=(Csew,inf)/(1 + k * HRTsew). This would apply to the uses releasing to municipal sewer.

After this WoE approach including both qualitative and quantitative information, the risk can be considered as acceptable for:

PT2: chemical toilets

PT11: closed systems with degradation (when no degradation is considered, the drainage leads to a slight excendance of PEC/PNECwater = 1.03; TAB ENV 126 could be applied as a risk mitigation measure).

PT11: open system with continuous and shock dosing

PT12: typical case scenario

5. ASSESSMENT OF EXCLUSION CRITERIA, SUBSTITUTION CRITERIA AND POP

Table 31: Assessment of exclusion criteria, substitution criteria and POP

Conclusion on exclusion criteria	
Conclusion on CMR	**Bronopol does not fulfil criteria (a) and (c) of Article 5(1). Due to a data gap on genotoxicity no conclusion can be drawn respecting to criterion (b)**
Conclusion on ED assessment	**No conclusion on ED properties for non-target organisms can be drawn**
Conclusion on PBT and vP/vB criteria	**Neither PBT nor vP/vB**
Conclusion on substitution criteria	**Not met for Bronopol**
Conclusion on LRTAP/POP assessment	**Not listed as POP**

A Assessment of intrinsic properties and effects of the active substance

A.1 General substance information

A.1.1 Identity of the Substance

Table 32: 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-nitro-1,3-propanediol
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 Details can be found in confidential Annex of the CAR/RAR.

Table 33: Structural formula

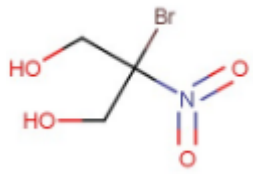
Structural formula


Table 34: Origin of the natural active substance or precursor(s) of the active substance

Origin of the natural active substance or precursor(s) of the active substance
n/a

Table 35: Method of manufacture

Method of manufacture
Details of the manufacturing process are reported in the confidential annex of the CAR/RAR.

A.1.2 Composition of the substance (reference specifications)

Table 36: 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-nitro-1,3-propanediol	≥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 37: 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
Sodium bromide	≤0.1 % w/w dry weight	≤0.1 % w/w dry weight	n/a	Repr. 2, STOT SE 3, STOT RE 2	A RAC Opinion is under discussion with the following proposal: Repr. 1B, Lact., STOT SE 3, STOT RE 1

Table 38: 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

Table 39: Concentration of constituents (main constituents, impurities, additives) in batches used for (eco)toxicity studies and proposed specification

	Batches used for (eco) toxicity studies [% w/w]				
	Active substance	**Impurity 1**	**Impurity 2**	**Impurity 3**	**Specification supported**
Relevant impurity (yes or no)					(yes/no*)
Proposed Specification [% w/w]					--

This information is included in the confidential annex of this CAR (Appendix VI).

The reference specification has been set or derived based on the available 5-batch analyses with the mean ± 3 standard deviations as it is described in the Guidance on the BPR, Volume I, Parts A+B+C (Version 2.1, March 2022) and an evaluation of the technical equivalence of the different sources of Bronopol as required under the BPR (Regulation (EU) No 528/2012) and following the Guidance on the BPR, Volume V, Guidance on applications for technical equivalence (Version 2.0, July 2018).

The content of the batches which were used in the tox and ecotox studies are listed in the confidential annex of the CAR, appendix VI, table 0-1. If specification is not supported by a batch used in a study, the constituent(s) which give concern is highlighted.

A.1.3 Physical and chemical properties of the active substance

Table 40: 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), 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 water, etc.) is not known. However, several other data sources indicate a melting point of Bronopol in the range of 130 °C, for instance:	[redacted] 2001 (A3_1_1-01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			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)
	Sample %-Through [in g/100 g] "P[redacted]": 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	[redacted] internal standard method [redacted], sieving method, purity min. 99.0%, GLP		[redacted] 2007 (B3.11_01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	Batch no. [redacted] 36; 22; 8; 3; 1 The estimated value of the measurement uncertainty is approx. +/- 26 % rel.			
	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) and the [redacted] study (2002) are almost identical for both given temperatures whereas in the study of [redacted] (2000a) only limit values could be derived for those two temperatures. Because of the similarity of values, both, the [redacted] and [redacted] study were considered to be accurate, reasonable and reliable. The value of the vapour pressure determined in the [redacted] study was used in the environmental risk assessment and is identical to the one listed in Appendix I: List of endpoints. This study was preferred only because of the higher purity of the test item.	[redacted] 2001 (A3.01.3_01)
	$4.92*10^{-3}$ Pa at 20 °C and $1.01*10^{-2}$ Pa at 25°C	Directive 92/69/EEC, A.4, OECD TG 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 m³/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	72 mN/m (at 20 °C and	Directive 92/69/EEC, A.5,	The test item is not surface-active.	[redacted] 2007

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
tension	1.0 g/L)	GLP, purity min. 99.0%		(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 TG 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. The value 304 g/L (pH 5; 20 ºC) is used in the environmental risk assessment and is identical to the one listed in Appendix I: List of endpoints.	[redacted] 2021b (no BPD-ID)
	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 Pow: −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 TG 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 Pow 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), [redacted] (2000b) and [redacted] (2007d) 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] 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], but [redacted] furthermore provides information on the log pow at different temperatures. Therefore, the value(s) of the [redacted] study is used in the risk assessment and are given in Appendix I: List of endpoints.	[redacted] 2007d (A3_9-02)
	log Pow: 0.15 temperature: 23 °C pH: 4.9	OECD TG 107, shake flask method, HPLC-UV detection, GLP, purity 99.6%	pH 4.9 was adjusted using a buffer consisting of Na_2HPO_4 and KH_2PO_4.	[redacted] 2021a (no BPD-ID)
	log Pow : 0.17	Directive 92/69/EEC A.8	The determination of the log Pow at pH 7 and pH 9 was not	[redacted] 2000b

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	temperature: 23 °C pH: 4.0 ratios tested: 1:0.25; 1: 0.667 and 1:4	(shake-flask method), GLP, purity: 98.7%	performed since the test substance is not stable under these conditions.	(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 TG 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 TG 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)
	[redacted] 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 involved in a fire.	Statement based on experience in use and handling		[redacted] 2005 (A3.10_02)
Reactivity towards container material	Not expected	Statement based on experience in use, non GLP	Crystalline Bronopol ([redacted]) 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	[redacted] 2000 (A3.17_01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
			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.	
	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 TG 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 ([redacted] and [redacted], 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.	
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	Directive 92/69/EEC, A.6, GLP, purity min. 99.0 %		[redacted] 2007 (A3.07_01)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
	°C: 1,500 mg/L Solubility in toluene at 30 °C: 2,500 mg/L			
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 41: 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 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	[redacted] Batch: [redacted] Purity 99.9 % GLP: No	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).	[redacted] 2015 (no BPD-ID)

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
	Directive 92/69/EEC A.14	[redacted] 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) Friction test: No explosion, no crepitation and no flames	[redacted] 2007 (A3.10_01)
	Directive 92/69/EEC A.14	Bronopol [redacted] 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*. 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>.	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
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.	
Flammable solids	Directive 92/69/EEC A.10	[redacted] 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 [redacted] 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	[redacted] 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	[redacted] [redacted] (Batch identification: [redacted]), purity min. 99.0%, GLP	No self-heating detected up to 400 ºC.	[redacted] 2007 (A3.10_01)
	Directive 92/69/EEC A.16	Bronopol [redacted] Batch: [redacted] Purity: 98.7%, GLP	No exothermic effects were recorded. 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
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	[redacted] 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 [redacted] 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 reference mixture (1.1 mm/s) Therefore, bronopol is not an oxidiser.	[redacted] 2000 (A3_11-01)
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.	

Hazard class / characteristics	Guideline and Method	Parameter(s)	Results / Waiver	Reference
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 [redacted] 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)
	Directive 92/69/EEC A.16	[redacted] (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	[redacted] 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

A.1.3.3 Explosives

Table 42: 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).	[redacted] 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. 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	[redacted] Batch: [redacted] Purity min. 99.0 % GLP: Yes	[redacted] 2007 (A3.10_01)
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 [redacted] 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. • 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 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) Test Series 3 • 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 bronopol is too insensitive for acceptance into Class 1 (explosives).	Bronopol Purity: 99.8% GLP: No	[redacted] 1992 (A3.11_02)

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. (Self-reactive substances and mixtures)	[redacted] Batch: [redacted] Purity: 99.6% GLP: No	[redacted] 2011 (no BPD-ID)
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)	[redacted] (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 [redacted] 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	[redacted] 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 [redacted] Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)
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.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.	[redacted] 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 [redacted] Batch: [redacted] Purity: 98.7% GLP: Yes	[redacted] 2000 (A3_11-01)

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) 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] and [redacted] (2015), *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^3/10 g 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).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.
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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.1.3.8 Flammable solids

Table 43: 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).	Bitterlich 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).	Heitkamp 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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.1.3.8.4 Self-reactive substances

Table 44: 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.		Keldenich 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 45: 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.	[redacted] (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 [redacted] 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.	[redacted] 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 [redacted] 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 46: 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.	[redacted] (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 [redacted] Batch: [redacted]1 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, this endpoint can also be waived accordingly.

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 47: 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 48: 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:	[redacted] Batch: [redacted] Purity min. 99.0% GLP: Yes	[redacted] 2007 (A3.10_01)

	The highest burning rate of 5.0 mm/s was determined 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 [redacted] 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. The tests specified in the CLP Regulation provide additional information on the packing group in case the tested substance turns out to be an oxidising solids. This is not relevant for bronopol as it was classified as "not oxidising solid" based on a negative result in the test according to A.17. Based on the available results for [redacted]/Bronopol ([redacted], 2007, BPD ID A3.10_01 with a burning rate much lower than the reference) it is very likely and thus can be assumed that the results of any tests according to O. 1 and O.3 will also result in a classification as "not oxidising solid". Therefore, the A.17 test is relevant for the CLP classification for bronopol. 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

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.
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').

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

Analytical methods for determination and identification of the active substance is presented below.

Impurities are considered confidential information, please refer to Appendix VI for further details.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 49: Analytical methods for detection and identification

Analyte (type of analyte *e.g.* active substance, metabolite/ degradant etc.)	Compartment	Linearity (r^2)	Specificity	Recovery rate (%)			Limit of quantification (LOQ), Maximum Residue Limits or other limits	Reference
				Fortification range / Number of measurements	**Mean**	**RSD**		
Active substance	Matrix: TGAI	0.9999	No inter-ference	60–100 g per 100 g; 6	99.46	0.39	Not applicable	[redacted] 2015a,b (BPD ID V1.2_06 and V1.2_07)
Active substance	Matrix: TGAI	0.9995	No interference	80.0–120.0 mg/L; 18	100.1	0.51	Not applicable	[redacted] 2000 (A4_01-01)
Active substance	Matrix: TGAI	1.0000	No interference	77–123 mg/L; 6	Not determined*	Not determined*	Not applicable	[redacted] 2006 (A4_01-02)
Active substance	Matrix: TGAI	>0.999	No interference	800 g/kg; 5 1000 g/kg; 5	100.8 100.9	0.26 0.36	Not applicable	[redacted] 2019 (no BPD-ID)
Active substance	Matrix: TGAI	Analyst 1: 0.999994 Analyst 2: 1.000	No interference	6.0–54 mg/25 mL; 3 concentrations, 6 samples, 2 analysts	Not determined	Not determined	Not applicable	[redacted] 2011 [redacted] 2012 [redacted] 2012a [redacted] 2012 (A4a_1-01)
Active substance	Matrix: TGAI	0.99990	No interference	60g/100g–100g/100g; 6	Not determined	Not determined	Not applicable	[redacted] 2001 (A4.1_01)

A4.1_01 ([redacted] 2001): The active ingredient is quantified by RP-HPLC by mixing with acetamidophenol-solution (serves as stabilizer), dissolving and diluting in eluent A (water/phosphoric acid = 2000:1 (v/v) + 5 mmol NaCl). The concentrations of bronopol are determined by liquid chromatography (reversed phase with gradient elution: acetonitrile/water/phosphoric acid/NaCl). The detection was carried out by means of UV spectroscopy at 214 nm.

A4.1a/01 ([redacted] 2011, [redacted] 2012, [redacted] 2012a, [redacted] 2012): The active substance is quantified by RP-HPLC-UV. Mobile phase, isocratic: 85% water containing 0.1% phosphoric acid / 15% acetonitrile. Injection volume: 5 µL. Flow rate: 1.5 mL/min. UV detection at 230 nm.

A4.1b/02 ([redacted] 2012b; [redacted] 2012c; [redacted] 2012d): The active substance is quantified by RP-HPLC-UV. Mobile phase, isocratic: 95/5/0.1 v/v/v water/acetonitrile/phosphoric acid (85%). Injection volume: 10 µL. Flow rate: 1.0 mL/min. UV detection at 214 nm.

A4.1/01 ([redacted] 2000): The active substance is quantified by RP-HPLC. Mobile phase: 50 mL acetonitrile, 950 mL water.

A2.7/01 ([redacted] 2006): The active substance is quantified by RP-HPLC. Mobile Phase: A: 990 mL demineralized water + 10 mL sulfuric acid $c(H_2SO_4)$ = 0.5 mol/l. B: 490 mL demineralized water + 500 mL acetonitrile + 10 mL sulfuric acid (H_2SO_4) = 0.5 mol/L. Injection volume: 10 µL. Gradient: 0-30 min: from 99% A to 1% A. Flow rate: 1.0 mL/min. UV detection at 225 nm.

V1.2_06 and V1.2_07 ([redacted] 2015a,b): The active ingredient is quantified by RP-HPLC-UV with gradient elution. The analytes are quantified by external standardisation. Bronopol standards are dissolved in an acetamidophenol (stabilizer) solution and diluted in the assay diluent. Wavelength λ = 214 nm. Column: Stainless steel column (150 x 4.6 mm), *e.g.* packed with Fluofix 120N, 5 µm, Wako; Stainless steel column (150 x 4 mm), *e.g.* packed with Aquasil C18, 5 µm, Thermo. Diluent: 1 mL of 50% sulfuric acid to 1 L eluent A. Eluents: A: 5 mM Na_2SO_4; B: 500 mL 5 mM Na_2SO_4 and 500 mL acetonitrile. Gradient: 0–20 min 100% A; to min 25: 70% A till min 40; to min 42: 100 A till min 55. Flow rate: 1 mL/min; Injection volume: 10 µL. Temperature: 25 °C.

[redacted] 2019 (no BPD ID): The active ingredient is quantified by RP-HPLC-UV in a mixture of water/acetonitrile/0.5 M aqueous sulfuric acid using a gradient programm over 20 min. Mobile phase: Eluent A: Water + 0.5 M sulfuric acid (99/1, v/v); Eluent B: Water + acetonitrile + 0.5 M sulfuric acid (49/50/1, v/v/v). Injection volume: 10 µL. Flow rate: 1 mL/min. UV detection at 225 nm.

Residue analysis

The analyte monitored for determination of residues is the parent compound bronopol. In the study of Wolf R (2007) additionally the possible degradation product of bronopol, 2-bromo-2-nitro-ethanol (BNE) was included besides bronopol itself. No BNE was detected in a drinking water or surface water sample in this study.

Table 50: Analytical methods for the determination of residues of Bronopol and relevant metabolites

Compartment	Analyte	Analytical method	Fortification range / Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination / Limit of quantification	Reference
						Range	Mean	RSD		
Water	Bronopol, residues	HPLC/MS by means of external calibration	0.1–10 µg/L (for drinking water and surface water) / 5 per concentration	0.05–1.1 µg/L r^2 = 0.9991 (drinking water) 0.05–1.1 µg/L r^2 = 0.9938 (surface water)	m/z 257.9 and 259.9 retention time 1.9 – 2.1 min	0.1 µg/L (d. w.) 91–109% 1 µg/L (d. w.) 83–115% 10 µg/L (d. w.)	97.0% 102.1%	9.8% 13.7%	LOQ=0.1 µg/L and LOD=0.05 µg/L (for drinking water and surface water)	[redacted] 2007 (A4.02c_01)

Compartment	Analyte	Analytical method	Fortification range / Number of measurements	Linearity	Specificity	Recovery rate (%)			Limit of determination / Limit of quantification	Reference
						Range	Mean	RSD		
						94–104% 0.1 µg/L (s. w.) 64-115% 1 µg/L (s. w.) 95–107% 10 µg/L (s. w.) 86-95%	99.3% 84.6% 102.1% 92.3%	3.8% 23.3% 4.8% 4.2%		
Water	Bronopol, residues	GC-ECD (deriviatisation)	0.05–0.50 µg/L / 10	≥0.991	No interference	73–96	86	9	LOQ=0.05 µg/L	[redacted] 2007c (A4_02_c_01)
Soil	Bronopol, residues	GC-ECD (deriviatisation)	0.05–0.50 mg/kg / 10	≥0.992	No interference	74–113	98	15	LOQ=0.05 mg/kg	[redacted] 2007a (A4_02_a_01)
Air	Bronopol, residues	GC-ECD (deriviatisation)	3.0–30 µg/m^3/ 10	≥0.994	No interference	74–99 75–91	84 83	11 8	LOQ=3.0 µg/m³	[redacted] 2007b (A4_02_b_01)

Soil

Supported uses for Bronopol based biocides are unlikely to give direct exposure to soil. Furthermore, chemical analysis of soil for Bronopol is scientifically unjustified as persistence or accumulation of Bronopol or its metabolites in soil is not expected in top or subsoil zones. However, a validated analytical method for determination of bronopol residues in soil is available. Separation and detection is achieved with GC-ECD, using two alternative colums (primary and confirmatory method). All validation parameter evaluated are within acceptable criteria: linearity, specificity, recovery (ranges between 70-110%), precision (RSD < 20%) and limit of quantification (LOQ = 0.05 mg/kg). The residues in treated samples were measured on dry soil weight basis.

Air

Supported uses for Bronopol based biocides are of no significant exposure to air. Bronopol is not recommended for use in aerosols and evaporation/volatilisation is not to be expected. An analytical method for determination of bronopol residues in air is available. Bronopol is extracted from air by sucking air through OVS sampling tubes equipped with two XAD-2 adsorbent sections. Separation and detection is achieved with GC-ECD. The method was shown to be well suitable for determination of Bronopol in air at ambient or enhanced conditions, with an LOQ of 3.0 µg/m^3 and a working range of 3.0 - 30 µg/m^3.

Water

For residue analysis in drinking water and in surface water a LC/MS method is available ([redacted], 2007). The aqueous sample was separated by reversed phase chromatography without any pre-treatment. Analysis was performed by electrospray ionisation mass spectrometry (ESI-MS) of the $(M+CH_3CO_2)^-$ -ions, which resulted from clustering of the analyte and the acetic acid used in the chromatographic system. Analysis was performed in the single ion monitoring (SIM) mode, the instrument was run in the single quadrupole mode. Evaluation was done by the method of external standard. SIM Ions m/z 257.9 and 259.9. Quantitation by MS/MS experiments could not be performed as under the conditions of MS/MS no suitable ions were formed from these clusters. The validation results confirm that the method is well suited to determine residues of bronopol in drinking water and surface water. All validation parameter are within acceptable criteria: linearity (r2 >0.99), specificity, recovery (ranges between 70-110%), precision (RSD < 20%) and limit of quantification (LOQ). The LOQ in drinking water and surface water is 0.1 µg/L.This information can be added to the dCAR.

Analytical method for determination of bronopol residues in water ([redacted], 2007c) is achieved with GC-ECD, using two alternative columns (primary and confirmatory method). The method was shown to be suitable for determination of bronopol in water with an LOQ of 0.05 µg/kg.

Animal tissues

Bronopol is not classified as very toxic.

As regards to the classification H301-Toxic if swallowed, bronopol is rapidly absorbed from the gastrointestinal tract and also rapidly eliminated predominantly via urine and to minor amounts via expired air and faeces, essentially independent of dose level.

While it is proposed to be classified as toxic by inhalation, the marked effects after inhalation exposure are expected to be mainly due to bronopol's irritation potential to mucous membranes, which is a local effect. The toxicokinetic profile of the substance is expected to be the same for this route of exposure, hence the observed toxic effects are of the same magnitude as for the oral route. For that reason, as it is rapidly eliminated from the body, analysis in body fluids is not necessary. Therefore an analytical method for body fluids and tissues for monitoring of systemic exposure is considered not to be required.

Food and feedstuffs

The product is not intended to be added to food and feedstuffs or be used in facilities during food processing.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.2 Effects against target organisms

Effects on target organisms

Bronopol effectively controls the growth of bacteria and is also effective against fungi including yeasts. According to [redacted] (1964) and [redacted] (1978) the anti-bacterial activity is greater than its anti-fungal activity. Examples of target organisms are listed in the table below.

Table 51: Target organisms

Property / Group of organisms	Species
Gram-negative	Genus *Pseudomonas*: *P. aeruginosa*, *P. fluorescens;* Genus **Proteus*: *P. inconstans, P. mirabilis, P. morganii, P. rettgeri, P. vulgaris;* Genus **Salmonella*: *S. enteritidis, S. gallinarum, S. ser. Dublin, S. ser. Heidelberg, S. typhimurium, S. typhosa;* Genus **Enterobacter*: *E. aerogenes, E. chloacae;* *Legionella pneumophila, *Shigella sonnei, *Klebsiella pneumoniae, *Escherichia coli, Alcaligenes faecalis, Aeromonas hydrophila*
Gram-positive	*Staphylococcus aureus, Streptococcus pyogenes, Enterococcus hirae, Bacillus subtilis* and *Corynebacterium sp.*
Fungi and yeast	Genus *Penicillium*: *P. brevicaule, P. roqueforti;* Genus *Trichophyton*: *T. metagrophytes, T. rubrum, T. tonsurans;* *Aspergillus niger, Chaetomium globosum, Cladosporium herbarum, Fusarium solani, Geotrichum candidum, Microsporum canis, Rhodotorula rubra, Stachybotrys atra, Trichoderma viride;* *Candida albicans*

Enteric bacteria are marked with an asterisk (*)

Function

Bronopol has bacteriostatic and bactericidal activity ([redacted] 1964; [redacted] 1973; [redacted] 1978). It controls the growth of microorganisms by first inhibiting and eventually killing actively growing cells by the generation of cell damage inducing oxygen radicals ([redacted] 1988). Where cells are not actively growing (*e.g.* spores) they may be killed, or they remain dormant within the product or process.

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; [redacted] 1988). 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).

[redacted] (1988) 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). 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.

Release of formaldehyde

Although Bronopol is often mentioned as a formaldehyde releaser, by definition the bactericidal activity of formaldehyde releasers is the result of the mode-of-action of formaldehyde. As detailed above, the efficacy of Bronopol is based on the reaction of the Bronopol molecule with thiol-groups and partly by the formation of oxygen radicals in microbial cells. Therefore, Bronopol should not be allocated to the class of formaldehyde releasers. Although laboratory studies have shown that Bronopol can hydrolyse to form formaldehyde in water, the hydrolysis rate is highly dependent on pH and the presence of other substances. On a per molecule basis, Bronopol can hydrolyse theoretically to form two molecules of formaldehyde. Thus, at a typical concentration of 300 ppm Bronopol (concentration range 100-500 ppm), in theory, a maximum of approximately 90 ppm formaldehyde could be formed if 100% of the Bronopol hydrolyses. However, in practice, the actual amount of formaldehyde formed is significantly less. This was also observed in the study by Kireche (2001)[4] where only low amounts of formaldehyde were produced from hydrolysis of Bronopol.

Due to its specific mode of action, Bronopol was not considered as formaldehyde releaser: "Concerning formaldehyde releasers identified by de Groot *et al.* 2009, Bronopol (CAS No 52-51-7) is not regarded as formaldehyde releaser according to the Member States Competent Authorities (see CA-Febr08-Doc.8.4 28th meeting of MSCAs for biocidal products)." "Bronopol can release low levels of FA due to its decomposition. These levels are not sufficient to work as biocide therefore Bronopol cannot be considered a FA releaser and should be removed from the list of substances" (comment to 28 BPR CA meeting -2008).

Field of use envisaged

Due to Bronopol's broad anti-microbial spectrum it can be used as disinfectant and preservative.

Bronopol is used in disinfection to control microbial growth in chemical toilets to reduce malodour.

Bronopol is also used as an industrial and cooling water preservative and slimicide to control microbially induced damage to plant / equipment, pipework and the proliferation of potentially pathogenic microorganisms during industrial and cooling processes.

[4] Kireche M, Peiffer JL, Antonios D, Fabre I, Giménez-Arnau E, Pallardy M, Lepoittevin JP, and Ourlin JC (2011) Evidence for Chemical and Cellular Reactivities of the Formaldehyde Releaser Bronopol, Independent of Formaldehyde Release. Chem. Res. Toxicol. 24(12), pp. 2115–2128.

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A.2.1 Intended uses

Table 52: Summary table of intended uses for PT2

Summary table of intended use	
Product Type	2 (Intended Use: Disinfection of chemical toilets)
Product description	Representative products with ≥ 99% active substance: [redacted]; [redacted]; [redacted]
Target organisms (including development stage)	Bacteria
Description of use(s)	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.
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.
Objects to be protected	Human
Concentration of product in the in-use formulation/product	-
Concentration of active substance in the in-use formulation/product	≥ 99%
Application rate(s)	16 – 80 mg/kg of matrix to be disinfected
Frequency of application	Single application
Season/period for use (where relevant)	Not relevant
Field of use (indoors/outdoors)	Indoors
Category(ies) of user(s)	Professional and non-professional users
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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 53: Summary table of intended uses for PT11

Summary table of intended uses	
Product Type	11 (Intended Use: Preservatives for liquid-cooling and processing systems)
Product description	Representative products with ≥ 99% active substance: [redacted]; [redacted]; [redacted]
Target organisms (including development stage)	bacteria, fungi and algae. Within this assessment report, only bactericidal efficacy is supported.
Description of use(s)	Bronopol is used as a cooling water preservative (*e.g.* in open and closed recirculating cooling systems). Preventive treatment with continuous dosing as well as curative treatment with shock dosing is intended. . Within this assessment report, both preventive and curative use are supported.
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.
Objects to be protected	Water matrix
Concentration of product in the in-use formulation/product	-
Concentration of active substance in the in-use formulation/product	≥ 99%
Application rate(s)	2 – 20 mg/L of water matrix to be preserved
Frequency of application	Continuous dosing or shock dosing
Season/period for use (where relevant)	Not relevant
Field of use (indoors/outdoors)	Indoors
Category(ies) of user(s)	Industrial and professional users
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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 54: Summary table of intended uses for PT12

Summary table of intended use(s)	
Product Type	12 (Intended Use: slimicide, paper industry)
Product description	Representative products with ≥ 99% active substance: [redacted]; [redacted]; [redacted]
Target organisms (including development stage)	Bacteria, fungi and algae. Within this assessment report, only bactericidal efficacy is supported.
Description of use(s)	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). Preventive treatment with continuous dosing as well as curative treatment with shock dosing is intended. Within this assessment report, both preventive and curative use are supported.
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.
Objects to be protected	Water circuits in paper machines
Concentration of product in the in-use formulation/product	-
Concentration of active substance in the in-use formulation/product	≥ 99%
Application rate(s)	10 mg/L of water matrix to be preserved
Frequency of application	Continuous dosing or shock dosing
Season/period for use (where relevant)	Not relevant
Field of use (indoors/outdoors)	Indoors
Category(ies) of user(s)	Industrial and professional users
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 55: 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, 11 and 12	[redacted]: 100% Bronopol	*Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	Inhouse method from [redacted]: Minimum Cidal Concentration (MCC) test.	Bronopol was manually pipetted to a pH-buffered solution containing the bacteria. After incubation at 37 °C 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.	A5.2-01 Supportive study
Inhibition of bacterial growth	PT 2, 11 and 12	[redacted]: 99.0% bronopol	Bacteria: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Alcaligenes faecalis*, *Corynebacterium sp*. Mould fungi: *Penicillium brevicaule*, *Chaetomium globosum*, *Aspergillus niger*, *Rhodotorula rubra*, *Fusarium solani* and *Geotrichum candidum*	Inhouse method No. [redacted] and [redacted] from [redacted]: Minimum Inhibitory Concentration (MIC) test	Bronopol was added to 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.*	The following MIC were 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.	A5.2-02 Supportive study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					candidum. 14 days for *P. brevicaule*, *C. globosum* and *A. niger* The number of replicates is no reported.		
Inhibition of bacterial growth	PT 2, 11 and 12	Bronopol	Bacteria (gram-positive): *Staphylococcus aureus,* *Streptococcus pyogenes,* *Corynebacterium pyogenes,* *Bacillus subtillis* Bacteria (75ran-negative): *Pseudomonas aeruginosa,* *Proteus vulgaris,* *Proteus rettgeri,* *Proteus morganii,* *Proteus inconstans,* *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*	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. 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.	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 Yeasts: ≥400 µg/mL	A5.1-01 Supportive study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			Trichophyton tonsurans *Microsporum canis* *Clasosporium herbarum* *Penicillium roqueforti* Yeast: *Candida albicans*				
Inhibition of bacterial growth	PT 2, 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 No controls are reported. Exposure time: 24 hrs The number of replicates is no reported.	The following MIC were determined: Gram-positive bacteria: 12.5-25 µg/mL Gram-negative bacteria: 6.25-12.5 µg/mL	A5.1-02 Supportive study
Inhibition of bacterial growth and bactericidal	PT 2, 11 and 12	Bronopol	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa*	**1)** Minimum Inhibitory Concentration (MIC) test	**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	**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.	A5.4-01 Key study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					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.	All controls shown growth.	
				2) Bactericidal effect for *E. coli*.	**2)** The bactericidal effect was determined for *E. coli* as a mean single survivor time (MSST) by the method of Mather from extinction data obtained by the technique of Berry & Bean using 25 replicates and 5 mL of neutralizing medium.	**2)** The MSST for *E.coli* was: 108 min for 700µg/mL; 3-5 h for 1000µg/mL; 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, 11 and 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	**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: 13 µg/mL. When the molar ratio of cysteine to Bronopol was 1:1, cysteine failed to	A5.4-02 Key study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					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 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 **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	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 pattern of inhibition. **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.	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					nutrient agar plates. Experiments were repeated at various temperatures at pH 7.0 and at various pH values at 35°C. Selected experiments were performed in the presence of catalase (100 U/mL) or superoxide dismutase (200 U/mL) or under anoxic conditions. Concentrations applied: unpreserved, 100 – 200 – 300 – 400 - 500 µg/mL Exposure time: 16 hours 3 replicates	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 dismutase. All controls shown growth.	
Inhibition of bacterial growth	PT 2, 11 and 12	Bronopol	Micro-organisms used are representative of those to be controlled in product types 2, 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,*	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.	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.	A5.3.1-01 Key study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			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*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium herbarum*, *Margarinomyces fasiculatis*, *Penicillium funiculosum*, *Stachybotrys atra*, *Trichoderma viride*		Water control plates were used. 2 replicates (where the replicates varied for any organism, the highest value obtained was quoted).		
Bactericidal	PT 2, 11 and 12	[redacted]: ≥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	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	200 ppm of [redacted] was shown to give a significant log reduction (>4 log) within 24 hrs for all organisms tested. 100 ppm of [redacted] demonstrated at least a 2 log reduction within 24 hours, except against *S. aureus*. Control sample showed growth.	A5.3.1-02 Supportive study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				performed according to EN 1040 and EN 1276 method.	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. 1 replicate.		
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' and the EPA Algicidal Test Guideline	A range of concentrations of bronopol were introduced into cultures of three freshwater algae to determine the algicidal concentration of bronopol. 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.	Bronopol was found to be algicidal against *Scenedesmus obliquus* between 10 ppm and 30 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.	A5.3.1_03 Key study

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; [redacted] 1988). 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).

[redacted] (1988) 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). 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). 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)[5]. 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. Catalytic oxidation of thiols in the presence of excess thiol can lead to an anoxic state. Under these conditions, the slower reaction with thiols, that consume bronopol, predominates. Consumption of bronopol by its reaction with thiol without the involvement of oxygene might lead to removal of bronopol and resumption of growth ([redacted] *et al.* 1988; [redacted] *et al.* 2004). But this phenomenon of reduced activity under the depletion of oxygene must not be confused with microbial biocide resistance, a genetically fixed insusceptibility of microorganisms to a biocide.

A relevant resistance mechanism based on inactivation of electrophilic biocides such as Bronopol by overproduction and/or excretion of sulfhydryl-containing compounds (*i.e.* cysteine or glutathione) is theoretically plausible but not yet found in real life. This is not surprising as such an overexpression is metabolically a very expensive resistance mechanism not being attractive for microorganisms as it would result in an extremely reduced fitness of the affected microbial population ([redacted] 2003).

The fact that no genuine in-use problems associated with the build-up of bronopol resistant microorganism are known although bronopol is used as a cosmetic and technical preservative since several decades, confirms the low likelihood of development of relevant bronopol resistance.

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)[5].

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 55.

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

[5] Anonymous 1992. Bronopol (BNPD) - Bronopol-[redacted]* BP, [redacted]* BT - A broad spectrum antibacterial agent. Technical Bulletin, issue 6, February 1992. [redacted], Nottingham, England.

microorganisms. The results showed that Bronopol was effective at concentrations ranging from 12.5 to 6400 parts per million (ppm) for the microorganisms tested. Bronopol was more effective versus bacteria, with range MICs of 12.5 to 400 ppm. The MIC values versus yeast were above 400 ppm and values versus mould ranged from 400 to 6400 ppm. Basic efficacy against the algae *Scenedesmus obliquus* at 10-30 ppm was also observed.

A.3 Assessment of effects on Human Health

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.

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". According to the CA-Febr08-Doc.8.4 28th meeting of MSCAs for biocidal products, 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. As formaldehyde has been evaluated under the Biocides Directive, please refer to the formaldehyde core dossier for specific information.

A.3.1 Toxicokinetics

Table 56: 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 the concentrations in whole blood to plasma was 3.4 and 2.3 in male and female rats, respectively.		[redacted] and [redacted] 1993 (A6.02_01)
US EPA Guideline No. 85-1, *in vivo* GLP Rel. 1	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:	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		[redacted] and [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
Key		Oral, gavage Observation period: 168 h (7 days)	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 (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.		[redacted]
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 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.		[redacted] 1993 (A6.02_03) [redacted]
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] and [redacted] 1993 (A6.02_04) [redacted]
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP)	Rat, CFY strain, male and female, 6 animals/sex for the metabolism study.	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1 mg/kg bw Route of administration:	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		[redacted] *et al.* 1974 (A6.02_05_a-d)

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	1 animal/sex for the biliary excretion study. 6 male rats from the metabolism study were used for autoradiography.	Oral, gavage Toxicokinetic was assessed by means of metabolism, biliary excretion and whole-body autoradiography.	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. 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.		[redacted]
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP) **Supportive**	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 Route of administration: Oral, gelatin capsules	Main excretion (81%) occurred via urine for both, male and female rats, with up to 64% of 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 tissue.		
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** (no guideline,	Rat, CFY, 6 animals	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg Dose: 1.2 mg/kg bw	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		

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
non-GLP, recoveries have a large variability) **Supportive**		(approx. 0.24 mg/cm^2 in the main study) Vehicle: Acetone Route of administration: Dermal, occlusive Exposure time: 5 days	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.		
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP) **Supportive**	Rabbit, New Zealand White 4 animals	TS: [2-^{14}C] Bronopol, specific activity: 21 µCi/mg 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	Up to 75% of the applied ^{14}C remained on the skin of the 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 GuidelineNo. 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 observed in skin and plasma. Assuming a body weight of 20 g per mouse, the applied dose accounts for about 75 mg/kg bw.		[redacted] and [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
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP, purity of non-labelled s.a. was not determined) **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] and [redacted] 1987 (A6.02_07) [redacted]
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP, purity of non-labelled s.a. was not determined) **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 examined for presence of bromide ion as a breakdown product of Bronopol.	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 bromide.	The capillary electrophoresis method used was especially developed for the purpose of bromide ion detection in rat urine (A6.02_08_b)	[redacted] and [redacted] 1993 (A6.02_08_a,b) [redacted]
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** (metabolism and elimination cannot be correctly characterized due to limitations in the information collected) **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:	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 elimination, multiple		[redacted] *et al.* 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
		Oral gavage Depending on the experiment urine, faeces, exhaled air, organs and/or blood were sampled.	applications did not reveal a relevant accumulation potential		
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** (low recovery does not allow the ADME of the substance to be correctly characterized) **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 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 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		[redacted] *et al.* 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
			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 TG 417, *in vivo* **GLP** **Rel. 2** (metabolite pattern and identification of metabolites was not performed) **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: 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) Route of administration: 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	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 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.		[redacted] and [redacted] 2007 (6_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
		collected and analysed at different time points.			
Non-guideline, *in vivo* **Non-GLP** **Rel. 3** (paper does not meet the minimum information requirements for regulatory purposes) **Supportive**	Rat and rabbit, CFY (rat), New Zealand White (rabbit), Sex and No. of animals not reported	TS: 2-^{14}C Bronopol (specific activity: 21 µCi/mg radiochemical 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)	Total dermal absorption including potential skin residues 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.		[redacted] *et al.* 1976 (A6_02-3)
Non-guideline, *in vivo* **Non-GLP** **Rel. 2** (no guideline, non-GLP, remarks) **Key**	Rat, Wistar Female Dermal: 7 Intravenous: 6	TS: 2-^{14}C Bronopol (specific activity: 15.4 µCi/mg, radiochemical purity: 99%) Vehicle: Dermal: acetone Intravenous: 0.9% NaCl solution Dose: Dermal: 1 mL/kg bw corresponding to 10 mg/kg bw (considering the range of body weights and the size of	After intravenous application about 85% 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.	Evaluation of the dermal absorption was difficult due to no skin wash and stripping was performed. In addition not all relevant data were determined in the same animals.	[redacted] and [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
		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) Route of exposure: Dermal or intravenous	The study authors 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] and [redacted] 1984 (B6.7_01_a) [redacted]
Investigation of the absorption Bronopol in volunteers following the	Human 10 volunteers, 5 males and 5 females	Test Substance: Bronopol Vehicle: Soltan cream (pharmaceutical formulation)	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%) whereas in males, recovery ranged between 57% and 67%. Mean Bronopol recovery in female		[redacted] *et al.* 1984 (B6.7_01_b) [redacted]

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
application to the skin of Soltan 3 cream containing 0.1% Bronopol **Non-guideline** **Non-GLP** **Rel. 3** **Supportive**		All items used for application and removal of the test 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. 4 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.	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 TG 417 (A6.02_01, A6.02_02, 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 (A6.02_01, A6.02_02).

Toxicokinetics observed after repeated oral application of Bronopol (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 (A6.02_03).

Doses of 1, 20 and 25 mg/kg bw (A6_02-1; 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 (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 (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 (A6.02_05c; 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 (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 ($\leq$ 1.3%) and expired air ($\leq$ 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 (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 (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 (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 (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 40.5% estimated from the amount of radioactivity detected in the site of application (59.5%). Since 7 animals were used in the study, k = 0.92 was applied. The standard deviation has been calculated from the deviation of the radioactivity measurements in the skin, so s = 3.4. In conclusion, the percutaneous absorption was estimated at 43.6%.

A.3.1.1.3 Metabolites

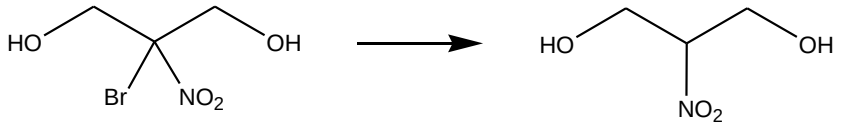
The urine samples obtained from the toxicokinetics studies reported in A6.02_01 to A6.02_03 had been used of identification of bronopol metabolites (A6.02_04). Urine samples collected during the first 24 h after application of [2-^{14}C]-bronopol were pooled within the same sex and group and analyzed 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 (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 (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 (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 (A6.02_08_a). That detection was based on a capillary electrophoresis method, which had been especially developed for this purpose (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 (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 werereached 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 43.6% 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

Value used in the Risk Assessment – Oral absorption	
Value	100%
Justification for the selected value	Based on the results of various studies, rapid and complete absorption (≥80% of applied dose) after oral exposure is concluded.

Value used in the Risk Assessment – Dermal absorption	
Value	43.6% 100% bronopol (powder): 10% (default value for solids) >5-30% bronopol: 10% (default value for concentrated water-based formulations) ≤5% bronopol: 50% (default value for diluted water-based formulation)
Justification for the selected value	Based on the results of various studies, it can be concluded that dermal absorption of 43.6% is a scientifically justified, conservative approach for Bronopol in the presence of potential penetration enhancers (diluted product). Considering the irritant nature of Bronopol 43.6% dermal absorption can also be considered as a worst case assumption for the neat material/undiluted product. However, for water-based mixtures, for which there is no information, default EFSA Guidance values should be applied.

Value used in the Risk Assessment – Inhalatory absorption	
Value	100%
Justification for the selected value	In the absence of (substance-)specific information, complete absorption (100%) is assumed for the inhalation route.

Conclusion used in the Risk Assessment – Distribution	
Conclusion	Bronopol is widely distributed, and highest tissue residues (beside blood) were seen in fatty tissue, skin and organs involved in excretion, *i.e.* kidney, liver, and lung.
Justification for the conclusion	Based on the results of various studies, it was shown that Bronopol is widely distributed before rapid elimination. No significant accumulation in tissues and organs has been observed. In rats and rabbits, fatty tissue, skin and organs involved in excretion, *i.e.* kidney, liver, and lung showed the highest concentration of residues.

Conclusion used in the Risk Assessment – Metabolism	
Conclusion	2-nitropropane-1,3-diol (NPD) is the main metabolite of Bronopol in urine and plasma samples in rats.
Justification for the conclusion	Based on the results of various studies, NPD was identified as the main metabolite, corresponding to up to 50% of the applied dose. It is detected at concentrations higher than Bronopol already in plasma at 0.25 h after application. Also, though 1 to 3 additional metabolites were found by chromatography, their molecule could not be resolved and thus, no further metabolites were identified.

Conclusion used in the Risk Assessment – Elimination	
Conclusion	Bronopol is rapidly excreted, mainly via urine (67-83%) whereas excretion via the faeces and exhaled air accounted for less than 10% each and played rather a minor role and there is no relevant accumulation.
Justification for the conclusion	Based on the available studies, it was shown that Bronopol is rapidly eliminated primarily via the urine (67-83%), and most part of urine excretion (64-81% of the total excretion) happened during the first 24 h after dosing. Total excretion was 88-98% within 72-120 h after application. After oral application of Bronopol maximum peak plasma concentrations of the metabolites were reached within 2 h. Mean half-life time was *ca.* 4 h for rats and dogs. No unchanged Bronopol could be detected in the plasma. There was no indication for a potential accumulation in the plasma.

A.3.2 Acute toxicity / STOT SE

A.3.2.1 Acute oral toxicity

Table 57: 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 TG 401 **GLP** **Rel. 1** **Key**	Rat Fischer 344 5 sex/group	Bronopol Doses: 100, 150 (females only), 200, 300 (males only) mg/kg bw Administration: single oral application (gavage)	Signs of gastrointestinal irritation (most of deaths within 24 hours) Dose (mg/kg bw) / Mortality (males) / Mortality (females) / Mortality (both) / Mortality (%) / Time of death 100 / 0/5 / 1/5 / 1/10 / 10 / Day 1 150 / - / 0/5 / 0/5 / 0 / - 200 / 2/5 / 3/5 / 5/10 / 50 / Day 1 300 / 5/5 / - / 5/5 / 100 / Day 1	LD50 male = 211 mg/kg bw LD50 female = 193 mg/kg bw		[redacted] 2001 (A6_01_1-1)
OECD TG 401 **GLP** **Rel. 2** (purity	Rat, Sprague-Dawley, male and female, 5	TS: Bronopol (purity not stated) Doses: 250, 500, 1000, and 2000 mg/kg bw	Signs of toxicity: hunched posture, piloerection, lethargy, ↓ respiratory rate. Dead animals showed abnormally red lungs	LD50 male = 273	Remark: male rats were more sensitive than the	[redacted] and [redacted] 1987 (A6.01.1_01)

<table>
<tr>
<td>not included)
Supportive</td>
<td>animals/ sex/group</td>
<td><u>Observation period</u>: 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</td>
<td>
<table>
<tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr>
<tr><td>250</td><td>1/5</td><td>2/5</td><td>3/10</td><td>30</td><td>1 to 2 h (day 0)</td></tr>
<tr><td>500</td><td>5/5</td><td>2/5</td><td>7/10</td><td>70</td><td>1 h – day 1</td></tr>
<tr><td>1000</td><td>5/5</td><td>5/5</td><td>10/10</td><td>100</td><td>1 to 4 h</td></tr>
<tr><td>2000</td><td>5/5</td><td>5/5</td><td>10/10</td><td>100</td><td>1 to 4 h</td></tr>
</table>
</td>
<td>mg/kg bw
LD50 female = 354 mg/kg bw
LD50 male, female = 305 mg/kg bw</td>
<td>females.</td>
<td>[redacted]</td>
</tr>
<tr>
<td>Similar to OECD TG 401
Non-GLP
Rel. 2 (non-GLP)
Supportive</td>
<td>Rat, Wistar, male and female, 10 animals/sex/group</td>
<td><u>TS:</u> Bronopol (99.0%)
<u>Doses:</u> 200, 280, 390, 550, 770 mg/kg bw
<u>Observation period</u>: 7 days
Oral</td>
<td>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.
<table>
<tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr>
<tr><td>200</td><td>3/10</td><td>0/10</td><td>3/20</td><td>15</td><td rowspan="5">Mortalities almost occurred within 19 h following treatment. Some additional mortalities were observed after 72 hours</td></tr>
<tr><td>280</td><td>3/10</td><td>5/10</td><td>8/20</td><td>40</td></tr>
<tr><td>390</td><td>8/10</td><td>7/10</td><td>15/20</td><td>75</td></tr>
<tr><td>550</td><td>7/10</td><td>7/10</td><td>14/20</td><td>70</td></tr>
<tr><td>770</td><td>10/10</td><td>10/10</td><td>20/20</td><td>100</td></tr>
</table>
</td>
<td>LD50 male = 307 mg/kg bw
LD50 female = 342 mg/kg bw</td>
<td>Male rats were more sensitive than the females.

Study was conducted before OECD TG 401 was available and before GLP was compulsory (devations: batch number was not recorded, no data on stability and homogeneity were given, no analytical details on the vehicle, records on the preparation of the vehicle were not kept, equipment maintenance and calibration records were not completed as required by current regulations,</td>
<td>[redacted] 1992 (A6.01.1_02)
[redacted]</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td></td><td></td><td></td><td>reagents were not always labelled with storage conditions and expiry dates, health status of the animals used in the study was not known, records on environmental conditions and animal husbandry procedures were not kept, diet, water and cage litter were not analysed for contaminant levels, formal SOP were not available).</td><td></td></tr>
<tr><td>Non-guideline
Non-GLP
Rel. 2 (non-GLP)
Supportive</td><td>Rat, Wistar, male, 10 animals/group</td><td><u>TS:</u> Bronopol (93.1%; impurities: sodium bromide 3.0%, [redacted], [redacted])
<u>Doses:</u> 36, 54, 80, 120, 180, 270, 400, 600 mg/kg bw
<u>Observation period:</u> 5 days
Oral</td><td>Signs: wheezing, gasping, nasal exudate, laboured respiration. Some animals were inactive and adopted a hunched body position.
Necropsy of dead animals revealed gastrointestinal irritation; adrenals appeared enlarged and darkened in some animals of the highest dose groups.
<table><tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (%)</th><th>Time of death</th></tr><tr><td>36</td><td>0/10</td><td>0</td><td rowspan="8">Deaths occurred within 5 d after dosing</td></tr><tr><td>54</td><td>0/10</td><td>0</td></tr><tr><td>80</td><td>3/10</td><td>30</td></tr><tr><td>120</td><td>3/10</td><td>30</td></tr><tr><td>180</td><td>5/10</td><td>50</td></tr><tr><td>270</td><td>5/10</td><td>50</td></tr><tr><td>400</td><td>5/10</td><td>50</td></tr><tr><td>600</td><td>8/10</td><td>80</td></tr></table></td><td>LD50 male = 254 mg/kg bw</td><td>Study was conducted before OECD TG 401 was available and before GLP was compulsory (devations: batch number was not recorded, no data on stability and homogeneity were given, no analytical details on the vehicle, records on the preparation of the vehicle were not kept, equipment maintenance and calibration records</td><td>[redacted] 1992 (A6.01.1_03)
[redacted]</td></tr>
</table>

					were not completed as required by current regulations, reagents were not always labelled with storage conditions and expiry dates, health status of the animals used in the study was not known, records on environmental conditions and animal husbandry procedures were not kept, diet, water and cage litter were not analysed for contaminant levels, formal SOP were not available).	

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 TG 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). 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 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 TG 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). 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). 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 TG 401 in rats oral LD50 values of 193 mg/kg bw (females) and 211 mg/kg bw (males) were determined for Bronopol ([redacted] 2001). 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 is classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008 (50 < LD50 ≤ 300 mg/kg bw). Bronopol is harmonised classified 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 is harmonised classified 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 is classified as Acute tox Cat.3 H301 under Regulation (EC) No 1272/2008.

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

Value used in the Risk Assessment – Acute oral toxicity	
Value	LD50 = 211 mg/kg bw (males), 193 mg/kg bw (females)
Justification for the selected value	Based on the results of the acute oral toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol. Bronopol is harmonised classified as Acute tox Cat. 4 H302, however as the most conservative values (211 mg/kg bw (males) and 193 mg/kg bw (females)), from a reliable guideline study are below the cut-off value for classification in Category 4, a higher, more severe classification is triggered. Therefore, Bronopol is classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008.

A.3.2.2 Acute dermal toxicity

Table 58: Summary table of animal studies on acute dermal toxicity

<table>
<tr><th>Method, Guideline, GLP status, Reliability, Key/supportive study</th><th>Species, Strain, Sex, No/Group</th><th>Test substance (including purity), Vehicle, Dose levels, Surface area</th><th>Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)</th><th>Value LD50</th><th>Remarks (e.g. major deviations)</th><th>Reference</th></tr>
<tr><td>OECD TG 402
GLP
Rel. 1
Key</td><td>Rat
Wistar
5 sex/group</td><td>Bronopol
<u>Dose</u>: 2000 mg/kg bw

single dermal application</td><td>2/5 females died. And showed discolorations of adrenal glands
Clinical signs: dermal irritation
<table><tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr><tr><td>2000</td><td>0/5</td><td>2/5</td><td>2/10</td><td>20</td><td>2 to 3 d</td></tr></table></td><td>LD50 > 2000 mg/kg bw</td><td></td><td>[redacted] 2000 (A6_01_2-1)
[redacted]</td></tr>
<tr><td>OECD TG 402
GLP
Rel. 1
Supportive</td><td>Rat, Wistar, male and female, 10 animals/sex/group</td><td><u>TS:</u> Bronopol (99.7%)
<u>Dose:</u> 2000 mg/kg bw
<u>Exposure:</u> 24 hours, semi-occlusive
<u>Observation period:</u>
18 days
Dermal</td><td>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.
<table><tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr><tr><td>2000</td><td>0/5</td><td>0/5</td><td>0/10</td><td>0</td><td>-</td></tr></table></td><td>LD50 male and female > 2000 mg/kg bw</td><td></td><td>[redacted] and [redacted] 2000 (A6.01.2_01)
[redacted]</td></tr>
</table>

<table>
<tr>
<td>No guideline
Non-GLP
Rel. 2 (no guideline)
Supportive</td>
<td>Male Boots-Wistar rats,
10 animals</td>
<td><u>Main study</u>:
Groups of ten male rats.
Dose: 25, 100, 400 and 1600 mg/kg, respectively.

Observation period: 14 days</td>
<td>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.
<table>
<tr><th>Dose (mg/kg bw)</th><th>Mortality (males)</th><th>Mortality (%)</th><th>Time of death</th></tr>
<tr><td>50</td><td>0/2</td><td>0</td><td>-</td></tr>
<tr><td>100</td><td>0/2</td><td>0</td><td>-</td></tr>
<tr><td>200</td><td>0/2</td><td>0</td><td>-</td></tr>
<tr><td>400</td><td>0/2</td><td>0</td><td>-</td></tr>
<tr><td>800</td><td>0/2</td><td>0</td><td>-</td></tr>
<tr><td>1600</td><td>2/2</td><td>100</td><td>Day 1</td></tr>
</table>
</td>
<td>1600 mg/kg bw</td>
<td></td>
<td>[redacted] 1992
(A6.01.2_02)

[redacted]</td>
</tr>
<tr>
<td>No guideline
Non-GLP
Rel. 2 (no guideline)
Supportive</td>
<td>Rats
2 male/group</td>
<td>64, 160, 400 and 1000 mg/kg bw in acetone</td>
<td>All rats given 1000 and 400 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.</td>
<td>Cannot be determined due to the small number of animals per group. Between 64 – 160 mg/kg bw</td>
<td></td>
<td>[redacted] 1967
(A6.01.2_03)

[redacted]</td>
</tr>
</table>

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 TG 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). 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] ([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] ([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 TG 402 (A6_01_2-1 [redacted] & [redacted], [redacted] 2000). 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] & [redacted] ([redacted] 2000) with a dermal LD50 of > 2000 mg/kg and A6.01.2_02 [redacted] [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 ($1000 < LD50 \leq 2000$ mg/kg), which it is supported by the harmonised classification under CLP Regulation (ATP 1 to CLP).

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

Bronopol is classified as Acute tox Cat. 4 H312 under Regulation (EC) No 1272/2008.

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

Value used in the Risk Assessment – Acute dermal toxicity	
Value	LD50 = 1600 mg/kg
Justification for the selected value	Based on the the study with the worst case value of LD50 of 1600 mg/kg, Bronopol is classified as Acute tox Cat. 4 H312.

A.3.2.3 Acute inhalation toxicity

Table 59: Summary table of animal studies on acute inhalation toxicity

<table>
<tr><th>Method, Guideline, GLP status, Reliability, Key/supportive study</th><th>Species, Strain, Sex, No/Group</th><th>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)</th><th>Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)</th><th>Value LC50</th><th>Remarks (e.g. major deviations)</th><th>Reference</th></tr>
<tr><td>OECD TG 403
GLP
Rel. 2 (only two dose levels available)
Key</td><td>Rat
Fischer 344
5 sex/group</td><td>Bronopol
<u>Nominal concentrations:</u>
0.42, 13.70 mg/L
<u>Analytical concentrations</u>:
0.12, 1.14 mg/L
<u>Exposure</u>: 4 hours (inhalation, nose only)</td><td>In the high dose level of 1140 mg/m³ (with a transient concentration of 3640 mg/m^3): 10/10 died.

Signs of respiratory irritation

<table><tr><th>Conc. (mg/L)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr><tr><td>0.12</td><td>1/5</td><td>0/5</td><td>1/10</td><td>10</td><td>Exposure period</td></tr><tr><td>1.14</td><td>5/5</td><td>5/5</td><td>10/10</td><td>100</td><td>Day 3</td></tr></table></td><td>0.12 mg/L <LC50 <1.14 mg/L</td><td>Significant difficulty in maintining a stable chamber aerosol concentration during the 1.14 mg/L exposure</td><td>[redacted] 2003 (A6_01_3-1)

[redacted]</td></tr>
</table>

<table>
<tr>
<td>Similar to OECD TG 403
Non-GLP
Rel. 2 (no guideline and non-GLP)
Key</td>
<td>Rat, Sprague-Dawley, male and female, 5 sex/group</td>
<td><u>TS:</u> Bronopol (99.7%)
<u>Nominal concentrations:</u>
0, 1.80, 2.59, 23.23 mg/L
<u>Analytical concentrations</u>:
0, 0.038, 0.089, 0.588 mg/L.
<u>Exposure:</u> 4 hours
Inhalation</td>
<td>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 direct contact with the test substance.
<table>
<tr><th>Conc. (mg/L)</th><th>Mortality (males)</th><th>Mortality (females)</th><th>Mortality (both)</th><th>Mortality (%)</th><th>Time of death</th></tr>
<tr><td>0</td><td>0/5</td><td>0/5</td><td>0/10</td><td>0</td><td>-</td></tr>
<tr><td>0.038</td><td>0/5</td><td>0/5</td><td>0/10</td><td>0</td><td>-</td></tr>
<tr><td>0.089</td><td>0/5</td><td>0/5</td><td>0/10</td><td>0</td><td>-</td></tr>
<tr><td>0.588</td><td>1/5</td><td>0/5</td><td>1/10</td><td>10%</td><td>Day 1</td></tr>
</table>
</td>
<td>LC50 for males and females > 0.588 mg/L (equivalent to > 588 mg/m³)</td>
<td>Study was conducted before GLP was compulsory.</td>
<td>[redacted] 1986 (A6.01.3_01)
[redacted]</td>
</tr>
</table>

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 TG 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/4h([redacted] 1986). 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 TG 403 exposing Fisher 344 rats to Bronopol at 120 and 1140 mg/m³ (nose only) for 4 hours ([redacted] 2003). 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 acute inhalation LC_{50} in rats is >0.12 mg/L but <1.14 mg/L, which corresponds to a classification of Acute tox Cat. 2 H330 under CLP Regulation. 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 is classified as Acute tox Cat. 3 H331 under Regulation (EC) No 1272/2008 (0.5 < LC50 ≤ 1.0 mg/L).

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 is classified as Acute tox Cat.3 H331 under Regulation (EC) No 1272/2008.

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

Value used in the Risk Assessment – Acute inhalation toxicity	
Value	0.588 mg/L/4h < LC50 < 1.14 mg/L/4h
Justification for the selected value	Based on the results of the acute inhalation toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol and the criteria for classification as Acute tox Cat. 3 H331 are met.

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 no classification and labelling for STOT SE 1 or 2 according to CLP criteria is 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 no classification and labelling for STOT SE 1 and 2 according to CLP criteria is 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 is required under Regulation (EC) No 1272/2008.Specific target organ toxicity – single exposure Category 3 (STOT SE 3)

Table 60: 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 TG 403 **GLP** **Rel. 2** (only two dose levels available) **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/m^3 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/m^3)	[redacted] 2003 (A6_01_3-1) [redacted]
Similar to OECD TG 403 **Non-GLP** **Rel. 2** (no guideline and non-GLP) **Key**	Rat, Sprague-Dawley, male and female, 5 animals/ sex/group	Bronopol (99.7%) Dose levels: 0, 1.80, 2.59, 23.23 mg/L (nominal) 0, 0.038, 0.089, 0.588 mg/L (analytical) Exposure: 4 h (inhalation, Nose-Head only)	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 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		[redacted] 1986 (A6.01.3_01) [redacted]

			(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 (A6_01_3-1 and 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 is harmonised classified as STOT SE Cat. 3 H335 under Regulation (EC) No 1272/2008 (ATP 1 to CLP).

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

Bronopol is harmonised classified as STOT SE Cat. 3 H335 under Regulation (EC) No 1272/2008 (ATP 1 to CLP).

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

Value used in the Risk Assessment – Acute systemic toxicity	
Value	Oral route: LD50 = 211 mg/kg bw (males), 193 mg/kg bw (females) Dermal route: LD50 = 1600 mg/kg Inhalation route: 0.588 mg/L/4h < LC50 < 1.14 mg/L/4h.
Justification for the selected value	Oral route: Based on the results of the acute oral toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol. Bronopol is harmonised classified as Acute tox Cat. 4 H302, however as the most conservative values (211 mg/kg bw (males) and 193 mg/kg bw (females)), from a reliable guideline study are below the cut-off value for classification in Category 4, a higher, more severe classification is triggered. Therefore, Bronopol is classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008. Dermal route: Based on the results of the acute dermal toxicity studies available for Bronopol, a low acute toxicity potential can be concluded for Bronopol. The worst case value of LD50 (1600 mg/kg) is considered for the classification. Therefore, Bronopol is classified as Acute tox Cat. 4 H312, which it is supported by the harmonised classification under CLP Regulation (ATP 1 to CLP). Inhalation route: Based on the results of the acute inhalation toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol and the criteria for classification as Acute tox Cat. 3 H331 are met.
Proposed classification	Oral route: Bronopol is classified as Acute tox Cat.3 H301 under Regulation (EC) No 1272/2008. Dermal route: Bronopol is harmonised classified as Acute tox Cat. 4 H312 under Regulation (EC) No 1272/2008 (ATP 1 to CLP). Inhalation route: Bronopol is classified as Acute tox Cat.3 H331 under Regulation (EC) No 1272/2008.

Value/conclusion used in the Risk Assessment – Acute local effects	
Value/conclusion	Oral route: Bronopol caused signs of gastrointestinal irritation in rats. Dermal route: Bronopol was shown to be irritating to skin of rabbits and cutaneous effects at the application site (white discoloration of the skin, erythema, edema, eczema-like skin change, scaling and crust formation) were shown in rats at 2000 mg/kg bw. Inhalation route: Bronopol was shown to cause nasal discharge, red staining and inflammation of the eyes and staining of the head, accompanied by swelling of the head, throat and/or the forepaws at 0.588 mg/L. Moreover, Bronopol caused bloody nose and mouth breathing (at 1140 mg/m^3 only) as well as labored breathing (at 120 and 1140 mg/m^3).
Justification for the selected value/conclusion	Oral route: No specific key value for the observed local effects can be established. However, local effects are sufficiently covered by the reference values for systemic effects on which the risk assessment is based. Dermal route: No specific key value for the observed local effects can be established. However, these observations are addressed by the classification as skin irritant Cat. 2 H315. support the classification risk assessment is based on systemic effects, whereby local effects are sufficiently covered by the reference values for systemic effects. Inhalation route: The observed clinical signs from the available acute inhalation toxicity studies are indicative of respiratory irritation and are addressed by the classification as STOT SE 3 H335.

A.3.3 Skin corrosion and irritation

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

Table 61: 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 TG 404 **GLP** **Rel. 1** **Supportive**	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) [redacted]
OECD TG 404 **GLP** **Rel. 2** (purity was not provided) **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] and [redacted] 1987 (A6.01.4_01) [redacted]

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 62: 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 and sensitisation.	[redacted] 1977 (A6.12.6_04)
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-	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	Anonymous 1984 (B6.6_01)

		year period.	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 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) 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). 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 TG 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, [redacted] 1977, [redacted] 1977, [redacted] 1978) revealed signs of skin irritation when exposed to Bronopol at 0.5 – 1% (at the induction phase). Additionally, medical surveillance data (Anonymous 1984), 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 is harmonised classified as Skin irritant Cat. 2 H315 under Regulation (EC) No 1272/2008 (ATP 1 to CLP).

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

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

Bronopol is harmonised classified as Skin irritant Cat. 2 H315 under Regulation (EC) No 1272/2008 (ATP 1 to CLP).

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

Conclusion used in the Risk Assessment – Skin irritation and corrosivity	
Value/conclusion	Bronopol causes skin irritation.
Justification for the value/conclusion	Based on the results of the available animal study data and the supporting human data for Bronopol, a skin irritating potential can be concluded. Bronopol is harmonised classified as Skin irritant Cat. 2 H315.
Proposed classification	Skin irritant Cat. 2, H315.

A.3.4 Serious eye damage and Eye irritation

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

Table 63: 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 TG 405 **GLP** **Rel. 1** **Key**	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) [redacted]

Draize Test **Non-GLP** **Rel. 2** (no guideline, non-GLP and purity was not provided) **Supportive**	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) [redacted]
Similar to OECD TG 403 **Non-GLP** **Rel. 2** (no guideline and non-GLP) **Supportive**	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) [redacted]

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 TG 405 ([redacted] 2000b) 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) 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) 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 TG 405 / Draize method and an acute inhalation study of Bronopol an eye irritating potential can be concluded. Bronopol is harmonised classified as Eye damage Cat. 1 H318 under Regulations (EC) No 1272/2008(ATP 1 to CLP).

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

Bronopol is harmonised classified as Eye damage Cat. 1 H318 under Regulations (EC) No 1272/2008 (ATP 1 to CLP).

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

Conclusion used in the Risk Assessment – Eye irritation and corrosivity	
Value/conclusion	Bronopol causes eye damage.
Justification for the value/conclusion	Based on the findings from eye irritation studies available for Bronopol and in accordance with the harmonised classification Bronopol is classified as Eye damage Cat. 1 H318.
Proposed classification	Eye damage Cat. 1, H318.

A.3.5 Skin sensitisation

Table 64: Summary table of animal studies on skin sensitisation

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 (*e.g.* EC3-value or amount of sensitised animals at induction dose)	Remarks (*e.g.* major deviations)	Reference
OECD TG 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: Induction: intradermal 1% Bronopol in PEG 400, epidermal 25% Bronopol in	Number of animals sensitised/total number of animals: 0/10 (treated) 0/5 (control) Non-sensitising		[redacted] 2001 (A6_01_5-1)

		PEG 400 Challenge: 12% Bronopol in PEG 400 day 1 (intradermal induction) day 8 (epidermal induction) day 22 (challenge) scoring 24h, 48h after challenge			
OECD TG 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) [redacted] [redacted] [redacted]
Guinea pig maximization test similar to OECD TG 406 **Non-GLP** **Rel. 2** (no guideline and non-GLP) **Key**	Guinea pig	Bronopol (100%) Dose levels: Induction: used as 0.02% (w/v; injection induction; solvent: 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.	After 3 challenges with Bronopol (0.4% aqueous solution), 2/10 animals were sensitised. 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.		[redacted] 1976 (A6.01.5_01) [redacted]

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Table 65: 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	Main effects, Observations	Reference
Study with volunteers Key	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 [redacted] 1977 (B6.7_04))
Study with volunteers Key	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	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 subgroup)	[redacted] 1998 (A6_01_5-5, A6.12.6_08)
Study with volunteers Key	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)

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

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, 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 induction	No. sensitised/total no.: 6/414 (for 5 of the positive 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%	[redacted] 1987 (A6_01_5-9, A6.12.6_07_b)
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 Result of 12% skin reactions were considered to be due to irritation according to a later publication by the same author (Maibach 1977)	[redacted] 1973 (A6_01_5-12)

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

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 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 (Maibach 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)
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 TG 406) ([redacted] 2001, [redacted] 1976). The earlier maximisation test ([redacted] 1976)was conducted according the acknowledged Magnusson and Kligman procedure which preceeded OECD TG 406 with 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), 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 TG 429 ([redacted] 2005). 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). 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, 1974). 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), 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) 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: 0.21% (clinically-relevant reactions), *i.e.* 17 of 8149 patients; [redacted] 1987: 1.4%, *i.e.* 6 of 414 patients; [redacted] 1997: 0.46%, *i.e.* 8 of 1996 patients). Furthermore, [redacted] (1986) 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). 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). A patch test was performed in humans ([redacted], 1997), 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). 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 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 Guinea pig maximisation tests (OECD TG 406) and an LLNA test (OECD TG 429). The earlier maximisation test ([redacted] 1976) 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) 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, no classification and labelling for skin sensitisation according to CLP criteria is 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 is warranted under Regulation (EC) No 1272/2008.

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

Conclusion used in the Risk Assessment – Skin sensitisation	
Value/conclusion	Bronopol is not skin sensitising.
Justification for the value/conclusion	Bronopol was found negative in two Guinea pig maximisation tests (OECD TG 406) and an LLNA test (OECD TG 429). In healthy volunteers, Bronopol did not induce skin sensitisation, while low incidences of dermal allergic responses were found in contact dermatitis patients.
Proposed classification	No classification proposed.

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 does not need to be taken into account.

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 66: 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. 1** **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 TG 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	NOAEL (local) = 0.025% (20.7/15.4 mg/kg bw/day for males/females) based on mild irritation in stomach at 0.05%	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)		[redacted] 2006 (A6_03_1-2)

		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 (systemic) = 0.05% (40.6/32.7 mg/kg bw/day for males/females)	high dose: as low dose + very slight multifocal hypertrophy of mucous cells in stomach (3 dogs)		

A sub-acute oral toxicity test was performed in Beagle dog (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.

% / Lesion	Males				Females			
	0	0.005	0.025	0.05	0	0.005	0.025	0.05
Nassal tissue - pharynx								
- Multifocal erosion of mucosa (slight)	0	1	0	0	0	0	0	0
- Subacute to chronic multifocal inflammation of mucosa (very slight)	0	2	2	2	0	1	1	1
Multifocal hypertrophy of the stomach mucous cells (very slight)	0	0	1	1	0	0	0	1

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

Value used in the Risk Assessment – Short-term oral toxicity	
Value/conclusion	NOAEL systemic = 0.05% corresponding to 40.6 and 32.7 mg/kg bw/day for male and female dogs, respectively; local = 0.025% corresponding to 20.7 and 15.4 mg/kg bw/day for male and female dogs, respectively. LOAEL systemic = 0.1%; local = 0.05% corresponding to 40.6 and 32.7 mg/kg bw/day for male and female dogs, respectively.
Justification for the value/conclusion	Based on the available 28-day repeated dose oral toxicity study, no treatment-related systemic toxicity has been observed in dogs exposed to Bronopol up to 0.05% and NOAEL and LOAEL are derived based on local effects (mild irritation) seen in the stomach.

A.3.7.1.2 Short-term dermal toxicity

Table 67: 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** **Non-GLP** **Rel. 2** (no guideline, non-GLP, no concentration with clear toxic effects was tested) **Key**	Rabbit, New Zealand White, male and female 5/sex/group	Bronopol (purity not stated) Concentrations: 0.2% and 0.5% in 2.5% methylcellulose (1 mL/kg bw) Treatment: Area: 10 cm^2 6 h/day, 7 d/week, non-occlusive Remark: 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.	NOAEL (local) = 0.2% Bronopol (2 mg/kg/day) based on skin irritation at 0.5% NOAEL (systemic) = 0.5% Bronopol (5 mg/kg/day)	No mortality and no signs of systemic toxicity. Skin irritation: Vehicle control (2.5% methylcellulose): Slight to well-defined erythema. 0.2% Bronopol: Findings similar control group. 0.5% Bronopol: 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.		[redacted] *et al.* 1973 (A6.03.2_01) [redacted]

Repeated dermal application of bronopol of unknown purity (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.

Value used in the Risk Assessment – Short-term dermal toxicity	
Value/conclusion	NOAEL systemic = 0.5% corresponding to 5 mg/kg bw/day for male and female rabbits; local = 0.2% corresponding to 2 mg/kg bw/day for male and female rabbits. LOAEL systemic = none in absence of any relevant indication for systemic toxicity; local = 0.5% corresponding to 5 mg/kg bw/day for male and female rabbits.
Justification for the value/conclusion	Based on the available 21-day repeated dose dermal toxicity study, no treatment related systemic toxicity has been observed in rabbits exposed to Bronopol up to 0.5% and NOAEL and LOAEL are derived based on dermal reactions (skin irritation) at the application site.

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 (A6_03_1-2 and A6.03.2_01), as well as sub-chronic oral toxicity studies (A6_04_1-1-3 and A6.04.1_01, 02) and chronic toxicity studies are available by oral (A6.07_01_a-f) and dermal (A6.07_02_a-e) route 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

Value used in the Risk Assessment – Short-term repeated dose systemic toxicity	
Value	Oral route: NOAEL = 0.05% corresponding to 40.6 and 32.7 mg/kg bw/day for male and female dogs Dermal route: NOAEL = 0.5% corresponding to 5 mg/kg bw/day in rabbits Inhalation: n.a.
Justification for the selected value	Oral route: Based on the available 28-day repeated dose oral toxicity study, no treatment-related systemic toxicity has been observed in dogs exposed to Bronopol up to 0.05% and key values are derived based on local effects (mild irritation) seen in the stomach. Dermal route: Based on the available 21-day repeated dose dermal toxicity study, no treatment related systemic toxicity has been observed in rabbits exposed to Bronopol up to 0.5% and key values are derived based on dermal reactions (skin irritation) at the application site. Inhalation route: No value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

Value/conclusion used in the Risk Assessment – Short-term repeated dose local effects	
Value/conclusion	Oral route: NOAEL = 0.025% corresponding to 20.7 and 15.4 mg/kg bw/day for male and female dogs, respectively Dermal route: NOAEL = 0.2% corresponding to 2 mg/kg bw/day in rabbits Inhalation: n.a.
Justification for the selected value/conclusion	Oral route: Local effects (mild irritation) were seen in the stomach of dogs treated with Bronopol. However, local effects are sufficiently covered by the reference values for systemic effects on which the risk assessment is based. Dermal route: Bronopol caused dermal reactions (skin irritation) at the application site of rabbits. However, these observations are addressed by (and support) the classification as skin irritant Cat. 2 H315. Inhalation route: no value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

A.3.7.2 Sub-chronic repeated dose toxicity

A.3.7.2.1 Sub-chronic oral toxicity

Table 68: 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 TG 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%) Dose levels: 0, 60, 250, 1000 ppm, equivalent to 0, 6.2, 24.3, 83.9 mg/kg bw/day (males) and 0, 6.8, 25.5 86.0 mg/kg bw/day (females) Treatment: daily in drinking water	NOAEL = 24.3/25.5 mg/kg bw/day for males/females LOAEL = 83.9/86.0 mg/kg bw/day for males/ females	low dose: no effects medium dose: no adverse effects (↓ water consumption, males; ↑ kidney weight, 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)		[redacted] and [redacted] 2001 (A6_04_1-1)
OECD TG 408 **90 days, oral (via drinking water)** **GLP** **Rel. 2** (see remarks) **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)	Range-finder for a 2-generation toxicity study with a smaller group size (8 instead of 10 rats/sex/ group) and with histopathological examinations limited to target organs identified in earlier studies with rats; no ophthalmology examinations were performed.	[redacted] *et al.* 2006 (A6_04_1-2)

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 TG 409 **90 days, oral (via drinking water)** **GLP** **Rel. 2** (high dose does not produce any adverse effect) **Supportive**	Dog Beagle 4 sex/group	Bronopol (99.9%) 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	NOAEL = 0.05% mg/kg bw/day for males/females LOAEL > 0.05% mg/kg bw/day for males/females	Low, medium and high dose: no treatment-related adverse effects		[redacted] 2007 (A6_04_1-3)
Similar to OECD TG 408 **13 weeks** **Non-GLP** **Rel. 2** (no guideline, non-GLP) **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 weight taken from among spare rats, which had been	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 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		[redacted] *et al.* 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
		treated with the appropriate dosage.		parameters affected by treatment during the first week, thereafter recovery was seen in the mid dose group. Ophthalmology: Inconspicuous. Haemaotology, clinical chemistry, 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 TG 409 **13 weeks** **Non-GLP** **Rel. 2** (no guideline, non-GLP) **Key**	Dog, Beagle, male and female, 3 animals/sex/group	Bronopol (99.2%) Dose levels: 4, 8 and 20 mg/kg 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.	NOAEL = 8 mg/kg bw/day LOAEL = 20 mg/kg bw/day	Mortality: None Clinical symptoms: Vomiting observed in all treatment 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		[redacted] *et al.* 1973 (A6.04.1_02) [redacted]

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
				female(s) of the low and high dose group, respectively. After 12 weeks of treatment, statistically significant decrease 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 female high dose group increased absolute liver and spleen weights were observed. No related gross pathological and histopathological changes were reported.		

Bronopol was given orally to groups of 20 males and 20 females at concentrations of 0, 20, 80 or 160 mg/kg bw/day during 90 days (A6.4.1_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. Groups of tubules wlth basophlllc epithellum and minimal mononuclear cell infiltration in the interstitial tissue were present in the kidneys in a proportion of males and in a few females from control and both treatment groups. In addition, small goups of tubules showing minimal dilatation were seen in the cortex in one rat of each group (in control group also). Foci of dystrophic mineralization were seen at the corticomedullary junction in a proportion of females from all groups and were noted within the lumina of occasional tubules in one (treated groups) or two (control group) rats. All these changes are seen commonly in laboratory rats and thus were considered not to be of toxicological significance. In 4 of the test animals, unusual renal changes for rats of this age were noted. In one rat of each treatment group distended tubules containing eosinophilic material were prominent and were associated with mononucleor cell infiltration in the adjacent interstitial tissue. In one rat of each treatment group (different from the previously mentioned) dilated tubules containing eosinophilic material were noted at the corticomedullcvy junction. These changes were not seen in control group and thus they were considered to be treatment related. Therefore, no NOAEL was established from this study as the low dose of 20 mg/kg bw/day showed clear evidences of renal damages.

Mortalities

Dose (mg/kg bw/d)	M	F	M + F
0	0/20	0/20	0/40
20	0/20	1/20	1/40
80	7/20	9/20	16/40
160	24/24*	25/25*	49/49**

Mean body weight gain (g)

mg/kg bw/d / Week	Males				Females			
	0	20	80	160	0	20	80	160
0 to 1	61	55	24***	9***	42	36	16***	20***
2 to 6	218	221	207	-	92	94	113**	-
7 to 13	133	134	133	-	52	55	57	-

**, p< 0.01; ***, p<0.001

Group mean food consumption (g/rat/week)

mg/kg bw/d / Week	Males				Females			
	0	20	80	160	0	20	80	160
1	141	134	73	42	123	117	70	75
2	109	164	152	0	129	117	121	0
3	206	211	207	0	149	154	145	0
4	206	210	197	0	150	153	161	0
5	185	185	173	0	139	125	134	0
6	172	173	184	0	123	116	105	0
7	179	178	180	0	132	141	129	0
8	183	181	176	0	130	130	128	0
9	180	184	173	0	139	136	141	0
10	172	172	171	0	121	124	134	0
11	195	211	208	0	140	146	140	0
12	174	165	180	0	113	119	111	0
13	167	177	161	0	124	121	129	0
Total	2329	2345	2235	42	1712	1699	1648	0
% ctrl	-	101	96	-	-	99	96	-

Group mean food conversion ratios

mg/kg bw/d / Week	Males				Females			
	0	20	80	160	0	20	80	160
1	2.3	2.4	3.0	4.7	2.9	3.3	4.4	3.8
2	2.9	2.9	3.2	-	4.6	3.9	3.9	-
3	4.2	3.5	4.5	-	5.5	5.7	3.9	-
4	4.1	4.7	4.1	-	12.5	11.8	8.9	-
1 - 4	3.3	3.3	3.8	-	5.1	5.1	4.9	-
5 - 8	6.4	7.0	5.8	-	10.9	12.5	9.9	-
9 - 12	10.8	10.0	10.3	-	20.5	15.9	21.9	-
1 -13	5.7	5.7	6.4	-	9.2	9.2	8.9	-

Haematological parameters

mg/kg bw/d / Parameters	Group mean value ± SD after 6 weeks				Group mean value ± SD after 12 weeks			
	Males		Females		Males		Females	
	0	80	0	80	0	80	0	80
Packed cell volume (%)	48±1.6	50±3.6	47±1.1	45± 2.5	49±1.7	48±2.0	49±1.5	48±1.2
Haemoglobin (g%)	15.4±0.7	15.4±0.8	14.7±0.3	14.4± 0.6	14.9±0.4	15.1±0.6	15.1±0.3	14.8±0.3
Red cell count (10^6/mm^3)	7.6±0.3	7.8±0.4	7.5±0.2	**7.3±0.2****	8.7±0.4	8.8±0.2	8.8±0.2	8.8±0.1
Corp. haemoglobin conc. (%)	32±0.6	**31±0.9***	32±0.4	32±0.7	30±0.5	31±0.7	31±1.0	31±04
Mean cell volume (μ^3)	63±2.5	64±3.1	62±2.9	62±2.8	57±3.6	55±1.7	55±1.5	55±1.3
White cell count (10^3/mm^3)	16.2±3.7	16.7±3.9	10.6±2.5	10.0±2.3	12.7±3.9	12.9±3.8	10.4±3.1	10.3±1.8
Neutrophils (10^3/mm^3)	1.3±0.7	**2.2±0.9***	1.0±0.5	1.1±0.5	1.0±0.7	**1.7±0.7***	0.9±0.7	1.1±0.4
Lymphocytes (10^3/mm^3)	14.6±3.7	14.4±3.4	9.6±2.3	8.8±2.3	11.7±2.9	11.1±3.4	9.4±2.6	9.1±1.8
Eosinophils (10^3/mm^3)	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1
Basophils (10^3/mm^3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Monocytes (10^3/mm^3)	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0

*, p<0.05; **, p<0.01

Clinical chemical parameters

mg/kg bw/d / Parameters		Group mean values and SD after 6 weeks				Group mean values and SD after 6 weeks			
		Males		Females		Males		Females	
		0	80	0	80	0	80	0	80
Plasma urea (mg%)		34±7.8	31±6.9	41±6.0	37±6.3	42±4.3	**37±3.2****	45±3.2	42±5.7
Plasma glucose (mg%)		113±7.1	**91±9.5*****	90±11.8	89±13.3	101±8.8	95±9.3	93±12.3	103±8.6
Total protein (g%)		5.9±0.3	5.9±0.2	6.0±0.2	6.0±0.3	6.9±0.2	**6.5**±0.3**	7.1±0.2	7.0±0.4
Serum proteins (%)	Alb.	3.3±0.2	3.2±0.2	3.3±0.2	3.3±0.2	3.5±0.3	3.5±0.2	3.9±0.2	3.7±0.3
	α1	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1	1.2±0.1	1.1±0.1	1.2±0.1	1.2±0.1
	α2	0.6±0.04	**0.7±0.08****	0.6±0.07	0.6±0.06	0.7±0.07	0.6±0.11	0.6±0.13	0.6±0.09
	β	0.8±0.14	0.8±0.13	0.8±0.10	0.8±0.08	1.0±0.11	1.0±0.08	0.9±0.07	1.0±0.11
	γ	0.2±0.10	0.3±0.09	0.4±0.10	0.4±0.16	0.5±0.11	**0.3±0.11****	0.4±0.15	0.5±0.14
Albumin/globulin ratio		1.25±0.14	1.17±0.20	1.22±0.10	1.20±0.11	1.04±0.16	1.16±0.14	1.23±0.15	1.15±0.17
Serum alkaline phosphatase (KA units)		62±13.0	57±8.4	38±3.6	43±8.4	34±6.5	32±5.1	21±2.8	23±3.5
Serum glutamic-pyruvic transaminase (mU/mL)		28±3.4	26±2.7	25±8.9	22±3.1	28±3.4	26±2.7	25±8.9	22±3.1
Sodium (mEq/l)		140±1.9	141±2.5	141±2.3	141±2.8	141±1.4	143*±2.2	143±2.0	143±2.0
Potassium (mEq/l)		4.3±0.3	4.6±0.4	4.4±0.3	4.4±0.2	4.6±0.3	4.6±0.3	4.2±0.2	4.4±0.3

**, p<0.01; ***, p<0.001

Organ weights as mean±SD (g)

Organ \ mg/kg bw/d		Males			Females		
		0	20	80	0	20	80
Body weight	-	529±43.2	525±49.6	**479**±56.3**	305±30.9	305±35.4	308±30.0
Brain	Abs.	2.0±0.13	2.1±0.10	2.0±0.10	1.9±0.08	1.9±0.10	1.9±0.11
	Rel.	38±3.2	40±3.4	**41*±4.7**	63±6.7	61±5.7	61±3.7
Pituitary (x10^3)	Abs.	17±3.0	18±2.5	15±2.7	18±2.7	17±2.9	19±3.9
	Rel.	0.3±0.06	0.3±0.04	0.3±0.06	0.6±0.10	0.6±0.10	0.6±0.11
Heart	Abs.	1.7±0.13	1.8±0.20	1.7±0.17	1.1±0.13	1.1±0.15	1.2±0.10
	Rel.	33±3.1	33±2.8	35±3.5	37±4.1	37±3.3	39±2.8
Liver	Abs.	21.9±2.26	23.4±3.37	19.9±3.07	12.0±2.08	11.9±1.55	13.1±2.11
	Rel.	414±27.8	**447*±56.6**	414±34.7	392±41.7	390±26.9	**423*±43.8**
Spleen	Abs.	0.9±0.17	0.9±0.16	0.8±0.15	0.7±0.14	0.6±0.08	0.7±0.13
	Rel.	17±3.1	17±3.1	17±2.8	22±3.6	21±2.9	22±3.2
Thymus	Abs.	0.7±0.18	0.6±0.16	0.6±0.15	0.5±0.10	0.4±0.10	0.5±0.15
	Rel.	14±3.4	12±2.7	**11*±1.4**	15±3.3	14±2.9	17±4.1
Uterus	Abs.	-	-	**-**	0.8±0.35	0.6±0.16	0.7±0.23
	Rel.	-	-	**-**	25±12.5	21±4.9	22±7.4
Kidney	Abs.	4.2±0.31	4.4±0.44	4.0±0.56	2.5±0.29	2.5±0.31	2.7±0.43
	Rel.	81±7.8	83±6.9	84±9.3	82±7.3	83±8.0	88±7.1
Thyroid (x10^3)	Abs.	23±4.4	**26*±5.0**	24±3.3	23±3.9	21±4.8	21±3.7
	Rel.	0.4±0.08	**0.5*±0.11**	**0.5*±0.05**	0.8±0.14	0.7±0.12	0.7±0.14
Adrenals (x10^3)	Abs.	67±13.2	65±10.1	75±12.8	72±14.3	71±13.8	80±9.3
	Rel.	1.3±0.26	1.2±0.22	**1.6**±0.38**	2.4±0.43	2.3±0.40	2.6±0.33
Testes	Abs.	5.4±0.32	5.3±0.34	**5.1**±0.43**	**-**	**-**	**-**
	Rel.	103±9.4	101±11.1	106±12.8	-	-	-
Ovaries (x10^3)	Abs.	-	-	-	88±17.9	91±14.5	**105*±21.9**
	Rel.	-	-	-	2.9 ±0.55	3.0±0.44	**3.4*±0.72**

Rel. = % bw x 100; *, p<0.05; **, p<0.01

A 90-day oral study in rats (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.

Mean body weight (g)

Week \ ppm	Males				Females			
	0	60	250	1000	0	60	250	1000
0	153	146	146	149	133	132	133	134
1	202	195	194	198	154	152	155	153
2	242	238	238	240	170	163	169	168
3	275	273	270	272	182	175	181	179
4	299	297	291	292	191	183	188	185
5	319	311	310	312	199	188	193	193
6	331	329	329	328	203	195	200	198
7	346	342	341	341	210	200	209	205
8	362	358	358	354	217	205	214	209
9	369	363	363	360	218	206	214	211
10	376	373	376	368	222	210	218	212
11	381	377	377	374	222	211	220	217
12	390	388	389	381	228	212	222	217
13	384	385	386	377	223	212	221	215
14	389	396	388	385	230	214	225	220

Group mean food consumption (g/day)

Week \ ppm	Males				Females			
	0	60	250	1000	0	60	250	1000
1	25	25	25	24	19	19	19	19
2	27	27	28	27	18	18	19	20
3	28	29	29	28	19	19	19	19
4	27	27	28	27	18	18	19	19
5	28	28	29	28	19	18	19	19
6	27	27	28	27	19	18	19	19
7	27	28	28	27	19	18	20	19
8	27	27	27	26	18	17	18	18
9	28	29	29	27	19	19	19	19
10	26	26	27	25	18	17	18	18
11	27	27	28	26	20	19	19	19
12	26	26	26	25	18	17	18	18
13	24	25	25	24	17	16	17	16
14	25	26	25	25	18	17	17	17

Kidney histopathology in terminal sacrifice group (K0)

Findings \ ppm	Males				Females			
	0	60	250	1000	0	60	250	1000
Basophilic tubules	7/10	9/10	9/10	9/10	3/10	3/10	2/10	6/10
- grade 1	6/10	9/10	7/10	7/10	3/10	2/10	1/10	6/10
- grade 2	1/10	-	2/10	2/10	-	1/10	1/10	-
Focal fibrosis	-	-	-	1/10	-	1/10	-	-
- grade 1	-	-	-	1/10	-	-	-	-
- grade 2	-	-	-	-	-	1/10	-	-
Hyal. Cast crot. Med	1/10	1/10	3/10	5/10	-	1/10	-	2/10
- grade 1	1/10	1/10	3/10	5/10	-	1/10	-	2/10
Hyl. Cast papilla	1/10	-	2/10	4/10	-	-	-	4/10
- grade 1	1/10	-	2/10	3/10	-	-	-	3/10
- grade 2	-	-	-	1/10	-	-	-	1/10
Hyaline inclusions	2/10	5/10	3/10	4/10	-	-	-	-
- grade 1	1/10	2/10	2/10	3/10	-	-	-	-
- grade 2	1/10	2/10	1/10	1/10	-	-	-	-
- grade 3	-	1/10	-	-	-	-	-	-
Mononuclear infiltr.	5/10	2/10	3/10	7/10	-	2/10	1/10	-
- grade 1	5/10	2/10	3/10	7/10	-	1/10	1/10	-
- grade 2	-	-	-	-	-	1/10	-	-
Pelvic dilation	-	-	1/10	-	-	-	-	-
- grade 2	-	-	1/10	-	-	-	-	-
Tubul. simple dilat.	-	-	-	2/10	-	-	-	-
- grade 1	-	-	-	2/10	-	-	-	-
Corticomed. mineral					-	1/10	-	-
- grade 1					-	1/10	-	-

Haematological parameters (week 13)

ppm / Parameters	Males				Females			
	0	60	250	1000	0	60	250	1000
Leucocytes (10^9/L)	11.21	12.17	11.59	11.46	7.90	8.11	7.89	7.79
Neutrophils (10^9/L)	0.89	1.45	1.22	1.02	0.69	0.67	0.97	0.91
Lymphocytes (10^9/L)	9.84	10.09	10.16	9.97	6.82	7.10	6.57	6.49
Monocytes (10^9/L)	0.23	0.32	0.28	0.21	0.20	0.15	0.16	0.18
Eosinophils (10^9/L)	0.16	0.19	0.19	0.17	0.15	0.14	0.13	0.16
Basophils (10^9/L)	0.03	0.03	0.03	0.03	0.02	0.02	0.01	0.01
Atypical leucocytes (10^9/L)	0.06	0.09	0.06	0.06	0.04	0.05	0.05	0.04
Diff	0	0	0	0	0	0	0	0
Erythrocytes (10^{12}/L)	9.1	9.23	9.13	8.84	8.25	8.33	8.34	8.46
Hemoglobin (g/L)	156	155	153	149	146	147	148	149
HCT (L/L)	0.469	0.466	0.461	0.455	0.430	0.431	0.438	0.438
MCV (fl)	51.6	50.5	50.5	51.5	52.2	51.8	52.5	51.8
MCH (pg)	17.2	16.8	16.7	16.8	17.7	17.7	17.8	17.6
MCHC (g/L ERY)	333	333	331	327	339	341	340	341
Thrombocytes (10^9/L)	1204	1165	1248	1208	1222	1228	1179	1122
Hepato quick (sec)	31.5	31.3	30.6	31.6	27.8	27.2	27.2	27.7
Microcytos	1	1	1	1	0	0	0	0
Hypochrom	0	0	0	0	0	0	0	0

Determination in liver tissues (week 14)

ppm / Parameter	Males				Females			
	0	60	250	1000	0	60	250	1000
N-DEM (mU/g)	164.2	149.6	143.0	144.9	81.5	76.0	73.0	79.6
O-DEM (mU/g)	10.5	12.9	10.4	11.0	9.3	11.7	12.5	13.1
Cytochrome P450 (nmol/g)	43.8	45.0	45.0	44.6	39.3	41.8	37.4	39.2
Triglycerides (µmol/g)	5.88	6.61	6.73	6.79	6.33	6.20	6.85	6.38

A second oral subchronic study was performed in rats under guidelines (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.

Mean food consumption ± SD (g/rat/week)

% / Day	Males				Females			
	0	0.025	0.075	0.15	0	0.025	0.075	0.15
1-4	24.5±1.9	24.4±1.6	23.1±1.5	17.9±4.2*	18.4±1.1	17.9±1.3	17.4±0.9	15.9±4.1
4-8	26.8±1.9	26.6±1.9	26.0±1.6	24.0±0.7*	18.7±1.2	18.4±1.6	18.7±1.3	18.6±0.9
8-15	28.1±1.9	28.1±1.8	27.8±1.9	26.2±2.7	18.8±1.6	18.3±1.3	17.7±2.0	18.3±1.8
15-22	28.6±1.9	28.5±1.5	28.5±2.7	26.8±3.3	20.0±2.3	18.7±1.6	18.3±2.4	18.6±2.1
22-29	29.1±1.7	29.4±1.5	29.1±3.4	26.5±3.7	19.9±2.2	19.5±1.3	19.2±1.3	19.3±2.4
29-36	29.4±1.7	29.0±1.5	29.7±3.7	26.9±3.4	20.3±2.2	20.4±2.5	19.5±4.2	20.0±2.7
36-43	30.6±2.0	29.8±3.0	29.1±3.2	26.8±3.8	19.7±2.3	20.4±2.6	20.7±3.9	19.6±3.3
43-50	29.8±1.6	29.5±2.9	28.9±3.2	28.3±3.5	20.5±2.3	19.8±1.4	19.9±1.5	20.2±3.2
50-57	28.9±1.8	28.8±3.3	28.7±2.8	28.1±4.0	19.8±2.3	19.4±1.1	20.1±2.3	19.5±1.8
57-64	28.8±1.6	29.1±2.9	28.5±2.6	27.9±4.1	19.2±1.8	18.8±1.3	20.8±5.4	19.0±2.5
64-71	29.1±1.6	29.0±2.1	28.8±2.3	27.6±4.2	19.1±1.7	19.1±1.3	18.7±1.3	18.8±2.4
71-78	28.7±1.5	27.8±1.4	27.2±1.1	27.4±3.9	19.1±2.2	18.8±1.7	18.9±1.2	18.2±2.4
78-85	27.5±1.4	27.2±1.7	26.1±2.0	25.8±3.1	17.9±1.6	18.2±1.9	18.6±1.2	17.9±1.3
85-90	27.8±1.9	27.4±1.5	27.3±2.6	26.4±2.7	19.4±1.8	18.1±1.3	18.1±1.2	17.3±1.5

*p<0.05

Mean water consumption ± SD (g/rat/week)

% / Day	Males				Females			
	0	0.025	0.075	0.15	0	0.025	0.075	0.15
1-4	34.5±2.7	31.4±1.9	27.3±2.1*	23.3±4.3*	26.4±2.3	27.0±4.6	24.2±5.1	18.5±7.2*
4-8	37.7±3.6	32.8±2.5	30.4±3.2*	32.5±7.7*	27.3±3.2	25.2±2.8	24.4±4.3	21.6±2.1*
8-15	39.3±2.7	35.4±2.9	32.7±3.6*	31.3±5.2*	26.7±3.1	25.0±2.9	22.8±3.0*	20.6±2.4*
15-22	42.0±4.7	36.6±3.1	32.8±3.2*	31.4±5.0*	28.4±3.9	26.1±4.6	23.4±3.7	23.8±5.1
22-29	43.6±6.8	36.6±3.0*	33.7±4.4*	32.0±4.7*	28.1±4.2	27.0±4.1	23.8±3.6	22.9±5.1
29-36	43.8±4.8	37.2±5.1*	34.6±4.2*	32.0±5.0*	28.5±4.9	28.3±6.3	21.4±2.5*	23.0±5.7
36-43	44.8±5.8	37.8±6.0*	34.4±4.8*	32.1±4.3*	28.9±5.4	27.2±4.7	25.9±4.8	22.9±5.5
43-50	42.8±4.1	36.5±5.7	33.8±4.7*	37.4±7.7	30.1±4.5	28.6±4.2	26.0±2.9	23.3±6.3*
50-57	42.6±4.2	37.0±5.2	34.5±4.8*	33.2±4.6*	28.2±4.4	28.2±4.3	25.8±5.3	22.1±4.4*
57-64	41.4±4.7	36.4±6.2	33.7±4.9*	33.2±6.4*	27.6±5.7	26.8±3.6	26.0±7.0	21.8±4.8
64-71	45.5±8.2	39.6±5.9	34.0±5.4*	32.4±5.6*	31.8±9.4	28.6±5.8	26.7±4.6	21.5±4.8*
71-78	41.8±6.0	34.5±4.0*	32.1±5.6*	32.7±6.1*	30.6±8.9	27.2±5.7	25.0±4.7	20.4±5.3*
78-85	41.1±7.8	34.2±5.5	29.3±3.2*	31.5±6.4*	29.7±7.2	26.7±5.8	25.9±3.9	19.7±3.5*
85-90	40.1±5.7	35.2±6.4	32.8±4.2	34.8±5.7	29.2±5.1	28.8±7.5	24.6±2.6	20.6±3.8*

Organ weight ± SD (g)

Organ / %		Males				Females			
		0	0.025	0.075	0.15	0	0.025	0.075	0.15
Adrenal glands	Abs.	0.05±0.01	0.06±0.02	0.06±0.01	0.059±0.008	0.08±0.01	0.072±0.006	0.07±0.01	0.08±0.02
	Rel.	0.009±0.002	0.011±0.003	0.011±0.002	0.011±0.001	0.027±0.006	0.025±0.004	0.025±0.003	0.027±0.004
Kidneys	Abs.	3.5±0.4	3.6±0.3	3.6±0.3	3.7±0.5	2.0±0.1	2.1±0.2	2.2±0.2	2.13±0.24
	Rel.	0.64±0.05	0.6±0.1	0.66±0.04	0.71±0.08	0.72±0.09	0.72±0.09	0.77±0.09	0.75±0.06
Liver	Abs.	15.7±2.3	15.4±1.2	15.4±1.4	14.7±2.3	7.8±0.4	7.7±0.6	8.0±0.9	7.60±1.07
	Rel.	2.8±0.3	2.8±0.2	2.8±0.1	2.8±0.1	2.8±0.2	2.7±0.2	2.8±0.2	2.7±0.2
Pituitary	Abs.	0.015±0.002	0.015±0.002	0.015±0.002	0.015±0.003	0.016±0.002	0.017±0.002	0.016±0.003	0.016±0.002
	Rel.	0.0028±0.0002	0.0027±0.0004	0.0027±0.0003	0.0029±0.0005	0.0055±0.0009	0.0059±0.0006	0.0057±0.0009	0.0056±0.0005
Salivary glands	Abs.	0.9±0.1	0.8±0.2	0.92±0.08	0.85±0.09	0.53±0.09	0.5±0.1	0.51±0.06	0.50±0.06
	Rel.	0.16±0.02	0.15±0.03	0.17±0.02	0.16±0.02	0.19±0.03	0.19±0.03	0.18±0.02	0.18±0.02
Testes	Abs.	3.7±0.3	3.8±0.3	3.5±0.3	3.7±0.3	-	-	-	-
	Rel.	0.7±0.1	0.7±0.1	0.64±0.07	0.71±0.09	-	-	-	-
Thymus	Abs.	0.4±0.1	0.4±0.1	0.32±0.07	0.34±0.06	0.33±0.04	0.9±0.1	0.34±0.09	0.28±0.05
	Rel.	0.07±0.01	0.06±0.02	0.057±0.008	0.06±0.01	0.12±0.02	0.10±0.02	0.12±0.03	0.10±0.01
Thyroid gland	Abs.	0.02±0.01	0.025±0.003	0.021±0.003	0.022±0.006	0.015±0.002	0.017±0.003	0.016±0.004	0.016±0.003
	Rel.	0.042±0.001	0.0044±0.0006	0.0039±0.0005	0.0042±0.0008	0.0054±0.0004	0.006±0.001	0.006±0.001	0.0055±0.0007
Epididymides	Abs.	1.4±0.1	1.4±0.1	1.4±0.1	1.4±0.1	-	-	-	-
	Rel.	0.26±0.02	0.25±0.01	0.25±0.02	0.27±0.03	-	-	-	-

Incidence and severity of findings in kidney:

Finding / %	Males				Females			
	0	0.025	0.075	0.15	0	0.025	0.075	0.15
No viable lesions	7/8	8/8	8/8	7/8	8/8	8/8	8/8	8/8
Cysts in left cortex	1/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Roughened surface in right kidney	0/8	0/8	0/8	1/8	0/8	0/8	0/8	0/8

In a 90-day drinking water study in dogs (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 (A6.4.1_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 relative 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 basis of increase in liver and spleen weight.

Number of doses per week which were followed by vomiting

Week	Control group (water)						20 mg/kg bw/day group					
	Males			Females			Males			Females		
	1	2	3	4	5	6	1	2	3	4	5	6
1	-	-	-	-	-	-	1	1	5	2	-	3
2	-	-	-	-	-	-	1	-	5	1	-	4
3	-	-	-	-	-	-	2	-	4	-	1	3
4	-	1	-	-	-	-	2	2	7	2	1	-
5	-	-	-	-	-	-	5	-	6	2	4	4
6*	-	-	-	-	-	-	2	-	-	-	2	-
7 - 13	-	-	-	-	-	-	-	-	1**	-	-	-
1 - 13	0	1	0	0	0	0	13	3	28	7	8	14

*, From week 6, dosing/feeding routine was changed and the dogs were fed prior to dosing.

**, One case of vomiting was reported for animal 3 during the week 7 –13 period.

Mean body weight gain

Test group	Males	Females
Control group	4367 g	3100 g
4 mg/kg bw/day	4583 g	5133 g
8 mg/kg bw/day	5366 g	3117 g
20 mg/kg bw/day	4700 g	5100 g

Main findings referring to organ weights

Test group		Absolute weight (g)		Relative weight (% bw)		
		Spleen	Ovaries	Liver	Spleen	Ovaries
Control		44.0	0.85	2.81	0.43	0.0092
4 mg/kg bw/day		47.6	0.79	2.89	0.43	**0.0072***
8 mg/kg bw/day		49.6	0.76	3.07	0.50	0.0088
20 mg/kg bw/day		**62.7***	**0.62***	**3.22***	**0.60***	**0.0060*****
LSD	**5%***	15.2	0.17	0.39	0.16	0.00098
	1%**	20.6	0.25	0.53	0.22	0.0014
	0.1%***	28.0	0.38	0.72	0.29	0.0021

LSD: least difference that has to exist between values obtained for treated groups and for control for significance at specified levels of probability.

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

Value used in the Risk Assessment – Sub-chronic oral toxicity	
Value/conclusion	NOAEL (rats) 24.3/25.5 mg/kg bw/day for males/females and (dogs) 8 mg/kg bw/day for males/females.
Justification for the value/conclusion	Key values are based on the most reliable, guideline-compliant sub-chronic repeated dose toxicity studies available for rats and dogs exposed to Bronopol. High doses of Bronopol mainly affected water and food consumption, body weight as well as kidney weights (including signs of nephropathy) in rats.

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 (A6_03_1-2 and A6.03.2_01), as well as sub-chronic oral toxicity studies (A6_04_1-1-3 and A6.04.1_01,02) and chronic toxicity studies are available by oral (A6.07_01_a-f) and dermal (A6.07_02_a-e) route 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 (A6_03_1-2 and A6.03.2_01), as well as sub-chronic oral toxicity studies (A6_04_1-1-3, and A6.04.1_01,02) and chronic toxicity studies are available by oral (A6.07_01_a-f) and dermal (A6.07_02_a-e) route 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

Value used in the Risk Assessment – Sub-chronic repeated dose systemic toxicity	
Value	Oral route: NOAEL (rats) 24.3/25.5 mg/kg bw/day for males/females and (dogs) 8 mg/kg bw/day for males/females. Dermal route: n.a. Inhalation route: n.a.
Justification for the selected value	Oral route: Key values are based on the most reliable, guideline-compliant sub-chronic repeated dose toxicity studies available for rats and dogs exposed to Bronopol. High doses of Bronopol mainly affected water and food consumption, body weight as well as kidney weights (including signs of nephropathy) in rats. Dermal route: No value exists since no data is available (please refer to waiving arguments above). Inhalation route: No value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Value/conclusion used in the Risk Assessment – Sub-chronic repeated dose local effects	
Value/conclusion	Oral route: not specified Dermal route: n.a. Inhalation route: n.a.
Justification for the selected value/conclusion	Oral route: Local effects following oral exposure to Bronopol are not specified in the available sub-chronic repeated dose toxicity studies but can be expected based on the acute as well as the short-term and long-term repeated dose toxicity studies. Nevertheless, possible local effects are sufficiently covered by the reference values for systemic effects on which the risk assessment is based. Dermal route: No value exists since no data is available (please refer to waiving arguments above). Inhalation route: No value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

A.3.7.3 Long-term repeated dose toxicity

A.3.7.3.1 Long-term oral toxicity

Table 69: 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** (no guideline, non-GLP, stability was monitored at a later time point) **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,	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, which is regarded as a spontaneous occurring lesion in the used rat strain.		[redacted] *et al.* 1976 (A6.07_01_a (reported in [redacted] 1978 and US EPA RED 1995)), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] and [redacted]

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
		drinking water		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).		1986 (A6.07_01_e), [redacted] 1985 (A6.07_01_f) [redacted]

In a no guideline study (A6.07_01), 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.

Mortality

Dose level	Main test series		Satellite test series	
	Males	Females	Males	Females
0 mg/kg bw/day	21/45	19/45	8/15	9/15
7 mg/kg bw/day	20/45	19/45	5/15	10/15
32 mg/ kg bw/day	20/45	22/45	7/15	8/15
142 mg/kg bw/day	**36/45*** **	**28/45***	12/15	10/15

*, $p<0.05$; ***, $p<0.001$

Group mean body weight gains (g)

mg/kg bw/d / Week	Males				Females			
	0	**7**	**32**	**142**	**0**	**7**	**32**	**142**
0 – 3	166	168	171	166	83	86	84	83
3 – 6	105	102	98	90	53	57	52	55
0 – 6	271	270	269	**256****	136	137	136	138
6 – 13	100	98	95	**73*****	45	47	47	**40****
0 - 13	371	368	364	**329*****	181	184	183	178
13 - 26	103	108	98	**52*****	45	47	**57***	38
26 – 52	110	107	**88****	**18*****	60	58	59	**28*****
52 – 78	98	74	**47*****	**36*****	69	80	83	**41****
78 – 104	40	7	23	**-104*****	35	39	0	**-7*****

*, p<0.05; **, p<0.01;***, p<0.001

Group mean food consumption (g/rat/week)

mg/kg bw/d / Week	Males				Females			
	0	**7**	**32**	**142**	**0**	**7**	**32**	**142**
1 – 13 (T)	2221	2208	2193	2169	1649	1678	1671	1657
1 – 13 (%)	-	99	99	98	-	102	101	100
14 – 26 (T)	2186	2164	2133	2000	1649	1648	1636	1589
14 – 26 (%)	-	99	98	**91*****	-	100	99	96
27 – 52 (T)	4233	4260	4171	**3954*****	3239	3185	3136	3116
27 – 52 (%)	-	101	99	93	-	98	97	96
53 – 78 (T)	4820	4687	**4477***	**4065*****	3702	3670	3637	3640
53 – 78 (%)	-	97	93	84	-	99	98	98
79 – 104 (T)	4797	4659	4649	4021	3936	3909	3737	3659
79 – 104 (%)	-	97	97	**84*****	-	99	95	93

Group mean water consumption (mL/rat/week)

mg/kg bw/d / Week	Males				Females			
	0	**7**	**32**	**142**	**0**	**7**	**32**	**142**
1 – 13 (T)	3769	2838	2435	1840	2574	2273	2014	1589
1 – 13 (%)	-	75	65	49	-	88	78	62
14 – 26 (T)	3493	**2595*****	**2196*****	**1657*****	2454	**2181*****	**1925*****	**1560*****
14 – 26 (%)	-	74	63	**47**	-	89	78	64
27 – 52 (T)	5829	**5010*****	**4338*****	**3264*****	5225	**4687*****	**4250*****	**3457*****
27 – 52 (%)	-	86	74	56	-	90	81	66
53 – 78 (T)	5997	5418	**4692*****	**4076*****	5661	5270	**4836****	**4279*****
53 – 78 (%)	-	90	78	68	-	93	85	76
79 – 104 (T)	6747	6397	6306	**3652*****	6695	6268	**5601***	**4021*****
79 – 104 (%)	-	95	93	**54**	-	94	84	60

*, p<0.05; **, p<0.01;***, p<0.001

Urinalysis

Week	Sex	Dose (mg/kg bw/d)	Volume (mL/rat)	PH (geometric means)	Specific gravity	Protein (mg%)
26	♂	0	6.0	6.9	1050	28
		142	5.4	6.7	1048	82
	♀	0	4.6	6.3	1044	15
		142	3.2	6.3	1050	33
52	♂	0	4.9	7.3	1050	17
		142	3.5	6.5	1055	65
	♀	0	4.5	6.8	1042	12
		142	2.7	6.5	1045	24
77	♂	0	7.6	7.0	1046	117
		142	9.0	6.7	1042	172
	♀	0	4.9	6.3	1043	111
		142	5.7	6.3	1042	174
103	♂	0	8.7	6.1	1041	62
		142	4.7	6.5	1045	148
	♀	0	11.3	6.3	1035	53
		142	7.3	6.4	1040	91

Incidence of heamoglobinuria

Dose (mg/kg bw/d)	Males			Females		
	Week 52	Week 77	Week 103	Week 52	Week 77	Week 103
0	1/10 (1/10)	4/10 (2/10)	5/10 (3/10)	0/10 (0/10)	0/10 (0/10)	1/10 (1/10)
7	-	4/10 (2/10)	3/10 (2/9)$^{+}$	-	1/10 (0/10)	0/10 (0/10)
32	-	9/10 (6/10)	3/10 (3/10)	-	3/10 (0/10)	0/10 (0/10)
142	5/10 (4/10)	5/10 (4/10)	10/10 (10/10)	3/10 (1/10)	8/10 (3/10)	2/10 (1/10)

In (), incidence of positives on repeat sampling; $^{+}$, one animal died before the test could be repeated.

Main treatment-related effects in the stomach:

	Control group				160 mg/kg bw/day			
	Males		Females		Males		Females	
	D	S	D	S	D	S	D	S
Thickening	0	0	0	0	2	3	1	2
Ulceration	1	0	1	0	0	1	0	4
Raised areas/excrescences	0	0	0	0	8	9	3	7

D, rats that died or were sacrificed in extremis; S, rats that were sacrificed at test ending

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.

Incidences and severity of findings in stomach

mg/kg bw/d	Male								Female							
	0		7		32		142		0		7		32		142	
Lesion	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Squamous cell papilloma	0	0	0	0	0	0	2 (1)	0	0	0	0	0	0	0	(1)	0
Papillomatous/marked epithelial hyperplasia	0	0	0	0	0	0	5 (3)	3	2	0	0	0	0	0	0	(2)
Moderate epithelial hyperplasia	1	0	0	0	1	0	3 (1)	4 (1)	1	0	0	0	1	0	2	3 (2)
Minimal focal epithelial hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Ulceration	1	0	0	0	0	0	5 (3)	5	1	0	0	0	0	0	(2)	6 (4)
Rats affected/Rats examined	1/4	0/24	1/1	0/25	1/1	0/21	8 (4)/8 (4)	7 (1)/8 (3)	2/2	0/26	1/1	0/26	0/0	0/23	(3)/(3)	8 (4)/17 (5)

(), satellite group animals

Abnormalities of the salivary glands:

mg/kg bw/d	Male				Female			
Lesion	0	7	32	142	0	7	32	142
Moderate squamous metaplasia in ducts	0	0	0	0	0	0	0	4
Minimal squamous metaplasia in ducts	0	1	4	4	0	0	0	1
Focal squamous metaplasia in ducts	2	1	4	5	1	1	2	3
Dilatation of the ducts	0	0	0	7	0	0	0	11
Acinar atrophy	3	4	6	11	0	0	1	8
Inflammation	2	5	12	9 (1)	2	1	3	9
Minimal epithelial hyperplasia	0	0	0	2	0	0	0	0
Rats affected/Rats examined	3/24	5/24	12/25	11 (1)/12 (1)	2/26	1/26	2/23	12/20

(), rat died during the treatment period

No human data on chronic oral toxicity is available.

Value used in the Risk Assessment – Long-term oral toxicity	
Value/conclusion	NOAEL = 7 mg/kg bw/day LOAEL = 32 mg/kg bw/day
Justification for the value/conclusion	In the available combined chronic toxicity/carcinogenicity study, systemic toxicity and non-neoplastic changes after long-term repeated exposure were evaluated in rats orally exposed to Bronopol via drinking water at 0, 10, 40, and 160 mg/kg bw/day. Mortality was

	significantly increased in the high dose group, and body weight gain and food consumption were significantly reduced in the mid and high dose group. 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 finding generally was associated with hyperplasia and ulceration of the non-glandular epithelium of the stomach. No such changes were seen in the control, low, and mid doses groups. The incidence and gravity of squamous metaplasia in the ducts of the salivary glands were increased in the mid dose group (males only) and in the high dose group (males and females) when compared to the control group. Squamous metaplasia was often associated with minimal mixed or chronic inflammatory cell infiltration, and with groups of atrophic acini. The affected glands often were dilated and displayed minimal epithelial hyperplasia. Though, squamous metaplasia in the salivary glands is regarded as a spontaneous changes occurring in the rat strain used; the treatment with Bronopol probably exacerbated the occurrence of this spontaneous lesion. Thus, the LOAEL and NOAEL for non-neoplastic changes after chronic oral exposure to Bronopol were considered to be 40 mg/kg bw/day and 10 mg/kg bw/day, respectively.

A.3.7.3.2 Long-term dermal toxicity

Table 70: 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 (no guideline, non-GLP, stability was monitored at a later time point) **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) Treatment: one application/day, 3	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 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.		[redacted] *et al.* 1975 (A6.07_02_a (reported in [redacted] 1978 and US EPA RED 1995)), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), [redacted] and [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
		days/week (*i.e.* on Monday, Wednesday and Friday) for 80 weeks		An increased incidence of skin papilloma was reported for the 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.		[redacted]

In an 80-week chronic toxicity and carcinogenicity study (A6.07_02), 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. 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.

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.

Value used in the Risk Assessment – Long-term dermal toxicity	
Value/conclusion	NOAEL = 0.2% corresponding to 20 mg/kg bw/day
Justification for the value/conclusion	In the available combined chronic toxicity/carcinogenicity study, systemic toxicity and non-neoplastic changes after long-term repeated exposure were evaluated in mice dermally exposed to Bronopol at 0, 0.2 and 0.5 %. Following dermal application reduced body weight development (males) and a slight hair loss were the only effects of treatment observed at the high dose level. The NOAEL of the mouse study therefore considered to be 0.2% Bronopol corresponding to approx. 20 mg/kg bw/day.

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 (A6.07_01_a-f) and dermal (1973A6.07_02_a-e) route 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

Value used in the Risk Assessment – Long-term repeated dose systemic toxicity	
Value	Oral route: NOAEL = 10 mg/kg bw/day Dermal route: NOAEL = 0.2% corresponding to 20 mg/kg bw/day Inhalation route: n.a.
Justification for the selected value	Oral route: The key values is based on the most reliable, most conservative chronic repeated dose toxicity study available for rats exposed to Bronopol. Dermal route: Following dermal application reduced body weight development (males) and a slight hair loss were the only effects of treatment observed at the high dose level. The NOAEL of the mouse study therefore considered to be 0.2% Bronopol corresponding to approx. 20 mg/kg bw/day. Inhalation route: No value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

Value/conclusion used in the Risk Assessment – Long-term repeated dose local effects	
Value/conclusion	Oral route: 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. Dermal route: Skin reactions were reported for rats treated with Bronopol. Inhalation route: n.a.

Justification for the selected value/conclusion	Oral route: However, local effects are sufficiently covered by the reference values for systemic effects on which the risk assessment is based. Dermal route: The observed skin reactions may be indicative of skin irritation properties of Bronopol. However, these observations are addressed by (and support) the classification as skin irritant Cat. 2 H315. Inhalation route: No value exists since no data is available (please refer to waiving arguments above).
Proposed classification	No classification proposed.

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 and liver are limited to rats, and are not present in dogs. Therefore, its relevance to humans is also limited and classification in this hazard class is not appropriate.

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 no classification and labelling for STOT RE 1 or 2 according to CLP criteria is 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 no classification and labelling for STOT RE 1 or 2 according to CLP criteria is required under Regulation (EC) No 1272/2008.

A.3.8 Genotoxicity / Germ cell mutagenicity

A.3.8.1 *In vitro*

Table 71: 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	Results (including cytotoxicity and +/- S9 mix)	Remarks (*e.g.* major deviations)	Reference
Bacterial gene mutation OECD TG 471, GLP Rel. 1 Key	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] 2000a (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 TG 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 was observed in the + or -S9 in any of the tester strains.		[redacted] and [redacted] 1986 (A6.06.1_01)
Chromosome aberration test OECD TG 473, GLP, Rel. 1 Key	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 %		[redacted] 2000b (A6_06_2-1)

			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 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 TG 473, **GLP** **Rel. 2** (no guideline) **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 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.	Addition of colcemid was not mentioned and no data were provided on the preparation of the cells for examination. Data from other studies referring to the stability were provided. The author reported that after a 2 h at 37 °C (pH 7.0 – 7.2), recovery was 10%; within the same sentence, a concentration of up to 4.2 µg/mL of formaldehyde was mentioned but without further information allowing allocation of this finding.	[redacted] and [redacted] 1986 (A6.06.2_01) [redacted]
Chromosome aberration test, similar to OECD TG 473 **GLP**	Formaldehyde (38%) <u>Concentrations</u>: 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 µg/mL	Human lymphocytes	**Negative** in the presence and **positive** in the absence of S9-mix. <u>Cytotoxicity</u> was not considered since tested formaldehyde concentrations were intended	This supports the assumption that the clastogenicity reported for Bronopol (see A6.06.2_01) rather was due to released formaldehyde	[redacted] and [redacted] 1986 (A6.06.7_01_a,b)

Rel. 2 (no guideline, no positive control to validate the study, cytotoxicity was not considered) **Supportive**	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.		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, 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).	than to the parent compound as such.	[redacted]
Gene mutation in mammalian cells OECD TG 476, GLP **Rel. 3** (huge variability in cytotoxicity and mutant frequency data intra- and interexperiment) **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, 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		[redacted] 2001a (A6_06_3-1) [redacted]

			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. 3** (modified guideline, huge variability in cytotoxicity and mutant frequency data intra- and interexperiment) **Key**	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: + S9: 4, 5, 6, 7, 8 µg/mL	Chinese Hamster V79 cells	**Negative** in the absence and presence of S9-mix. Positive and negative controls were included in the test. Cytotoxicity: -S9 mix: 16 µg/mL. +S9 mix: 8 µg/mL.		[redacted] and [redacted] 1986 (A6.06.3_01) [redacted]

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 (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 (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 (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. No significant increases in mutant frequencies were reported in the same concentration range without S9 and significant increases in the presence of S9 were seen only at the top concentration showing excessive cytotoxicity, *i.e.* cloning efficiencies <10% *vs*. controls. It should be noted that triplicates (-S9) or duplicates (+S9) have very different values, although within each experiment there is a positive trend in the increase in cytotoxicity with increasing dose. However, this trend is not clear with increasing mutant frequency.

An *in vitro* gene mutation test was assayed in Chinese hamster V79 cells (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. Although interpreted as positive (without S9), evident cytotoxicity was observed, the findings were not reproducible between two runs and the significant increases in mutation frequencies observed were not concentration-dependent. Furthermore, negative control levels (13.3 mutants/10^6 cells) of the first run were evidently higher than in the respective controls of the second run (3.3 mutants/10^6 cells). These control values were only slightly below the range of the significant increases observed after a.s. treatment (14.5-24.3 mutants/10^6 cells) in the second run, which was considered positive. Furthermore, the maximum rates observed after a.s. treatment were only marginally above historical spontaneous mutation rate ranges (0.4 - 19.3 or 0.4 - 12.5 mutants/10^6 cells in negative or vehicle controls without S9, respectively). When compared to the positive control results (1,094-6,785 mutants/10^6 cells), the significant mutation frequencies can be considered marginally low and without clear dose-response relationship. Results obtained in the presence of S9, showed a concentration related significant increase in mutant frequencies (2.8 to 16.1 mutants/10^6 cells) in the first run, however, these were not found significant in the second run at comparable test concentrations. Furthermore, the negative control values varied comparably between both runs (*i.e.* 0.7 *vs*. 5.4 mutants/10^6 cells) and the frequencies were comparable with historical spontaneous mutation rates (0.4 – 17.9 or 0.4 – 12.4 mutants/10^6 cells in negative or vehicle controls with S9, respectively). In this study a relationship between dose and cytotoxicity and mutant frequency was impossible to establish as the values are very different both within the same experiment and in comparison to the parallel experiment in both conditions (-/+S9).

Bronopol was tested in the chromosomal aberration assay using human lymphocytes (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 (A6.10_01_a, A6.10_01_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-nitro-1,3-propanediol.

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 (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 (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. *In vivo*

Table 72: 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**	**Main effects, Observations (specify regarding dose and sampling time)**	**Remarks**	**Reference**
Mouse bone marrow micronucleus test OECD TG 474, GLP Rel. 2 (outdated guideline: see remarks) **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) PCE:NCE Ctrl. – (0 mg/kg bw) = 1.24 3 mg/kg bw = 1.12 6 mg/kg bw = 1.35 12 mg/kg bw = 0.76 Ctrl. + (20 mg/kg bw) = 0.88	(2x) 12 mg/kg bw considered as i.p. MTD in male mice. I.p. administration is not acceptable according to the recent guidelines. 2000 PCEs/animal have been abalysed whereas 4000 PCEs are recommended.	[redacted] 2001b (A6_06_4-1)
Mouse bone marrow micronucleus test OECD TG 474, GLP Rel. 1 Key	Bronopol (98.6%) Vehicle: Distilled water Doses: 0, 25 (males only), 50, 100, 200 (females only) mg/kg	Mouse CD-1 6 sex/sampling time Treatment: two oral applications (24 h between applications)	**Negative** (no increase in micronucleated polychromatic erythrocytes) PCE:NCE Ctrl. – (0 mg/kg bw) = 0.63	(2x) 100 and 200 mg/kg bw considered as MTD in male and female mice	[redacted] and [redacted] 2001 (A6_06_4-2)

Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Main effects, Observations (specify regarding dose and sampling time)	Remarks	Reference
	bw	Sampling: 24 h after last treatment	25 mg/kg bw = 0.60 50 mg/kg bw = 0.62 100 mg/kg bw = 0.67 Ctrl. + (200 mg/kg bw) = 0.47		[redacted]

Mouse micronucleus assay
OECD TG 474,
GLP
Rel. 2 (outdated guideline: see remarks)
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
		
		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.
			Negative
			(the number of polychromatic erythrocytes containing micronuclei was within the range of the negative control)
			
			PCE:NCE
			Ctrl. – (0 mg/kg bw) = 1.05 (M) // 0.96 (F)
			80 mg/kg bw = 0.15 (M) // 0.25 (F)
			160 mg/kg bw = 0.25 (M) // 1.24 (F)
			Ctrl. + (75 mg/kg bw) = 0.06 (M) // 0.42 (F)
			
			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.
				160 mg/kg bw was considered the MTD.
				
				Deviations: only 2 doses have been tested and the MTD might be to high considering the mortality observed within 48 h (1/3/sex died within 48 h). Only 1000 PCEs/animal have been abalysed whereas 4000 PCEs are recommended.
					[redacted] and [redacted] 1986 (A6.06.4_01)
					
					[redacted]

UDS test in rat hepatocytes **OECD TG 486,** **GLP** **Rel. 1** **Key**	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) [redacted]
UDS test in rat hepatocytes **OECD TG 486,**	Bronopol (99.5%) Doses: 0, 60, 150	Rat, Wistar, male 5 (controls) and 25 (mid and high dose) males/sampling time	**Negative** at 2-4 h and 12-14 h. (no increase in net nuclear	150 mg/kg bw considered as oral MTD in male rats	[redacted] and [redacted] 1998

Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Main effects, Observations (specify regarding dose and sampling time)	Remarks	Reference
GLP **Rel. 1** **Supportive**	mg/kg bw	Treatment: i) Oral, gavage, single application. Sampling times: 2-4 and 12-14 h	grain counts)		(A6.06.5_01) [redacted]
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** (no guideline, non-GLP, purity and stability were not provided) **Supportive**	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 and dead implants were recorded	**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 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		[redacted] 1974 (A6.06.6_01) [redacted]

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study	Test substance (including purity), Vehicle, Doses	Relevant information about the study	Main effects, Observations (specify regarding dose and sampling time)	Remarks	Reference
			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 (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. It should be noted that the PCE/NCE ratio only decreases in females at the low dose and in males at both doses while in females at the high dose an increase is observed.

Two MN test (with i.p. and oral application) were performed in mice. In the i.p. MN test (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 PCE was not affected, so bronopol was not considered genotoxic. No indications for clastogenicity *in vivo* were seen. With both administrations, no dose-related decrease in PCE:NCE is observed, occurring only at the highest dose.

In an oral mouse bone marrow micronucleous test (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. However, the PCE/NCE ratio is not shown in this study, so it is impossible to know whether the active substance has reached the bone marrow.

In an *in vivo* genotoxicity study (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) (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*.

The UDS test is generally able to detect substances, that induce *in vivo* gene mutations, because this assay is sensitive to some (but not all) DNA repair mechanisms. It serves as an indicator test which detects predominantly substance-induced longpatch repair (20-30 bases) and - which a much lower sensitivity - shortpatch repair (1-3 bases). Thus, the assay contains some remaining uncertainties in detecting point mutations determined in the HPRT assays, which were repaired especially by short-patch base excision repair (BER). However, the assays need to be assessed together with the overall dataset, in particular other genotoxicity and carcinogenicity data available. According to the REACH guidance document R7a, a liver UDS test may be adequate to waive the information requirement for an *in vitro* mammalian cell gene mutation test (justified on a case-by-case basis), when it can be reasonably assumed, that the liver is a target organ. The following reasoning supports, that liver cells – representing the target cells - can be considered exposed to a.s. in the UDS assays performed:

- In A6.06.5_01 Wistar rats were tested via single oral gavage administration at dose of 60 and 150 mg/kg bw in water. Doses were selected based on a pretest at 100, 125, 150 and 200 mg/kg bw, showing clinical signs (abnormal gait, abnormal breathing) that led to sacrifice in extremis at 150 and 200 mg/kg bw. Thus, 150 mg/kg bw was selected as MTD for the main assay. In the main assay, no mortality was seen but abnormal gait and abnormal breathing at 150 mg/kg bw group and at 60 mg/kg bw group (single animal) indicated systemic toxicity after administration, which supports bioavailability and indicates exposure of hepatocytes.
- In A6_06_5-01 Fischer 344 rats were treated via single oral gavage administration at 100 and 200 mg/kg bw in water. Doses were selected based on a toxicity pre-test (application of 100, 200, 300, 500 and 600 mg/kg bw), where deaths were observed at ≥300 mg/kg bw in both sexes and additionally 1/3 females at 100 and 200 mg/kg bw, 200 mg/kg bw was considered to represent the MTD. In the main test, mortalities and clinical signs (laboured breathing/gasping, squinted eyes and wet fur around the mouth) at 200 mg/kg bw and reduced activity at 100 mg/kg bw), indicate uptake/systemic availability and, thus, also support target organ exposure.
- Toxicokinetic data provide further proof for the uptake of a.s. after single (or repeated) oral administration (even at lower concentrations, than in the UDS assays performed), since a.s. was found to be efficiently absorbed, widely distributed with highest levels detected in liver and kidney (involved in absorption and excretion) and rapidly metabolised and eliminated. In a toxicokinetic study with CD rats, 14C-Bronopol was administered once via oral gavage at 10 mg/kg bw in water containing 0.4% hydroxyethylcellulose and the distribution and excretion was examined over a period of 7 d. Bronopol was extensively absorbed with a total recovery of at least 75%. The recovery of radioactivity in tissues and carcass was greater than in plasma, with the highest concentrations of radioactive material being found in the liver (0.108 - 0.236 µg/g). Radiolabeled material was also detected in bone samples (0.093 - 0.137 µg/g). Single application of 50 mg/kg bw 14C-Bronopol via oral gavage to CD rats, confirmed the high total recovery (absorption) and higher radioactivity in tissues/carcass than in plasma, however, highest concentrations were reported for the lung, fat and kidney tissue. Although lower, due to the overall rapid absorption, metabolism and excretion/elimination, in which liver is (besides kidney) the major responsible organ, the presence of the labeled material in liver (0.665 - 0.690 µg/g) and bone (0.761 - 0.861 µg/g) tissues was still evident. Overall, significant Bronopol uptake after oral gavage application and exposure of target organs such as liver and the bone marrow can be confirmed at even lower doses than

administered in the UDS assay by the toxicokinetic data available. In conclusion, the liver cells were clearly exposed in the dose range that was used in the UDS test and, thus, the indicator test could be used as clear indicator of absent genotoxicity.

A dominant lethal assay was performed in mice (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.

Conclusion used in Risk Assessment – Genotoxicity *in vivo*	
Conclusion	It cannot be concluded that Bronopol does not induce genotoxicity *in vivo*.
Justification for the conclusion	Results from all three *in vivo* test systems give no indication that Bronopol causes genotoxic effects *in vivo*, both in somatic and germ cells. However, UDS assays are known to be not sufficiently reliable due to their lack of sensitivity (only large repair areas can be detected).

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 (A6_06_1-1, A6.06.1_01).

Results of mammalian cell gene mutation tests in Chinese hamster V79 cells according to OECD TG 476 (A6_06_3-1) exhibited equivocal results in the presence and positive results in the absence of metabolic activation. In another study (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 (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 (A6.10_01_a and A6.10_01_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-nitro-1,3-propanediol. 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 A6.06.7_01_a and A6.06.7_01_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 (A6.06.7_01_a and A6.06.7_01_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.

According to the BPR, a gene mutation study in bacteria, a chromosomal aberration study and a gene mutation study in mammalian cells are required to study genotoxicity *in vitro*. If any of these studies give a positive result, a corresponding follow-up with an appropriate *in vivo* study should be performed.

The most recently conducted mammalian cell gene mutation study was weak positive. This result was followed-up *in vivo* with a UDS assay, which when the eCA started the evaluation (03/2022), was still included in the BPR guidance for information requirements (Part A). The validity and sensitivity of the UDS test was scientifically questioned. This is in line with the new versions of the BPR application guidance Part A for HH and BPR (29/03/2022 and 15/04/2022, respectively), the UDS assay was removed. The guidance came into force on 29/09/2022, and the eCA submitted its finalized dCAR to the Applicant for its 30-day commenting period on 14/02/2023. During the trilateral discussions prior to the Working Group (23 June 2023), the eCA strongly advised the Applicant about a possibility to conduct the corresponding study to study *in vivo* mutagenicity (comet or TGR) noting that the acceptability of the UDS was questioned in the RCOM by the CAs.

The Applicant decided to follow a WoE approach, as the timeframe did not allow the applicant to perform the appropriate study, which was not accepted by the WG. A data gap for a reliable *in vitro* gene mutation test in mammalian cells was identified by the Human Health WG. Given the too short timeframe of 10 days following the WG to perform the study, no request for a new study was made by the WG.

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, no classification and labelling for mutagenicity is 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 no classification and labelling for Genotoxicity/ Germ cell mutagenicity according to CLP criteria is required under Regulation (EC) No 1272/2008.

A.3.8.2.4 Overall conclusion on genotoxicity related to risk assessment

Conclusion used in the Risk Assessment – Genotoxicity	
Conclusion	It cannot be concluded that Bronopol is not genotoxic *in vivo*
Justification for the conclusion	No mutagenic activity of Bronopol was observed in bacterial strains of *S. 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* (CAT). The slightly positive effects observed in a CAT 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. Results from three *in vivo* test systems give no indication that Bronopol causes genotoxic effects *in vivo*, both in somatic and germ cells. However, UDS assays are known to be not sufficiently reliable due to their lack of sensitivity (only large repair areas can be detected). According to the BPR, a gene mutation study in bacteria, a chromosomal aberration study and a gene mutation study in mammalian cells are required to study genotoxicity *in vitro*. If any of these studies give a positive result, a corresponding follow-up with an appropriate *in vivo* study should be performed. The most recently conducted mammalian cell gene mutation study was weak positive. This result was followed-up *in vivo* with a UDS assay, which when the eCA started the evaluation (03/2022), was still included in the BPR guidance for information requirements (Part A). The validity and sensitivity of the UDS test was scientifically questioned. This is in line with the new versions of the BPR application guidance Part A for HH and BPR (29/03/2022 and 15/04/2022, respectively), the UDS assay was removed. The guidance came into force on 29/09/2022, and the eCA submitted its finalized dCAR to the Applicant for its 30-day commenting period on 14/02/2023. During the trilateral discussions prior to the Working Group (23 June 2023), the eCA strongly advised the Applicant about a possibility to conduct the corresponding study to study *in vivo* mutagenicity (comet or TGR) noting that the acceptability of the UDS was questioned in the RCOM by the CAs. The Applicant decided to follow a WoE approach, as the timeframe did not allow the applicant to perform the appropriate study, which was not accepted by the WG. A data gap for a reliable *in vitro* gene mutation test in mammalian cells was identified by the Human Health WG. Given the too short timeframe of 10 days following the WG to perform the study, no request for a new study was made by the WG.
Proposed classification	No classification proposed.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12	

A.3.9 Carcinogenicity

Table 73: 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	Reference
Non-guideline 104 weeks (oral via drinking water) Non-GLP Rel. 2 (no guideline, non-GLP, stability was monitored at a later time point) **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 drinking water Duration of exposure: 104 weeks	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 carcinogenicity was observed up to the highest tested dose level)	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, 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. Overall result: Bronopol tested orally in rats over a period of 104 weeks was not carcinogenic.		[redacted] *et al.* 1976 (A6.07_01_a (reported in [redacted] 1978 and US EPA RED 1995)), [redacted] 1993 (A6.07_01_b), [redacted] 1985 (A6.07_01_c), [redacted] 1998 (A6.07_01_d), [redacted] and [redacted] 1986 (A6.07_01_e), [redacted] 1985 (A6.07_01_f) [redacted]
Non-guideline 80 weeks	Mouse CFLP mice	Bronopol (98 - 102%)	NOAEL = 0.2% (20 mg/kg	Systemic toxicity: A slight but statistically significant decrease in mean		[redacted] *et al.* 1975

(dermal) **Non-GLP** **Rel. 2** (no guideline, non-GLP, stability was monitored at a later time point) **Supportive**	Males and females 52/sex/dose	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	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)	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 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. Overall result: A carcinogenic potential could not be evidenced for Bronopol under the test conditions used.		(A6.07_02_a (reported in [redacted] 1978 and US EPA RED 1995)), [redacted] 1986 (A6.07_02_b), [redacted] 1992 (A6.07_02_c), [redacted] 1998 (A6.07_02_d), [redacted] and [redacted] 1973 (A6.07_02_e) [redacted]

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) 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). 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), 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.

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, no classification and labelling for carcinogenicity is 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, no classification and labelling for carcinogenicity is required.

A.3.9.4 Overall conclusion on carcinogenicity related to risk assessment

Conclusion used in Risk Assessment – Carcinogenicity	
Value/conclusion	NOAEL for systemic toxicity = 10 mg/kg bw/day (rats) and 20 mg/kg bw/day (mice) NOAEL for carcinogenicity > 160 mg/kg bw/day (rats) and > 50 mg/kg bw/day (mice) Based on the available data and in the absence of genotoxicity *in vivo*, Bronopol is considered to be non-carcinogenic.
Justification for the value/conclusion	Carcinogenicity of Bronopol was assessed after oral and dermal exposure to rats and mice, respectively. Incidental findings were reported in both studies, which occurred in both treated and untreated animals, and showed no dose-response relationship. With no treatment-related significant increase in neoplastic findings after chronic oral and dermal exposure of rats and mice, Bronopol is considered not carcinogenic.
Proposed classification	No classification proposed

A.3.10 Reproductive toxicity

A.3.10.1 Sexual function and fertility

Table 74: 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 TG 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	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 reproductive: increased postimplantation loss, decreased gestation survival only at the high dose F1/F2 pups: No effects observed		[redacted] *et al.* 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
		Exposure period: P1/P2 adults: 10 weeks pre-mating, up to 2 weeks mating, gestation, lactation				
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 generation (F0) until 33 to 47 days following weaning of the	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-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). Pathological findings: 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 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		[redacted] 1987 (A6.08.2_01_a, b) Range-finding study: [redacted] 1986 (A6.08.2_03) [redacted]

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
		F2.		group showed significant decreases in absolute kidney and liver weights. Reproduction parameters: Effects reported at the highest test 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** (non-GLP, outdated guideline: see remarks) **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 Exposure period: 19 weeks	NOAEL (F0, F1), systemic toxicity = 40 mg/kg bw/day NOAEL, reproduction and fertility = 40 mg/kg bw/day NOAEL, development = 40 mg/kg bw/day	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 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.	The duration of the pre-mating treatment period for the males was 63 d (below 70 d, which covers a rat spermatogenic cycle). Only 2 treatment groups were used; no clear data on (histo)pathological examinations were provided.	[redacted] and [redacted] 1973 (A6.08.2_02) [redacted]

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) (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 (A6.08.2_01_a) and in a one-generation study following the FDA guideline available at that time (A6.08.2_02).

In the two-generation study (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 without statistical significance 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.

Body and organ weights values

mg/kg bw/d / Organ		0	22.5	55.2	147
F0 - Males					
Body weight (g)		567 ± 33	609 ± 53	615* ± 45	552 ± 40
Adrenal	Abs. (mg)	64 ± 11	57 ± 8	58 ± 7	60 ± 8
	Rel. (%x10^3)	11.4 ± 2.1	9.3* ± 1.3	9.5* ± 1.6	10.9 ± 1.5
Heart	Abs. (g)	1.66 ± 0.19	1.72 ± 0.17	1.66 ± 0.15	1.60 ± 0.17
	Rel. (%x10)	2.94 ± 0.41	2.04 ± 0.29	2.72 ± 0.33	2.90 ± 0.31
Kidney	Abs. (g)	4.14 ± 0.32	4.21 ± 0.29	4.33 ± 0.40	4.15 ± 0.52
	Rel. (%x10)	7.32 ± 0.57	6.96 ± 0.75	7.05 ± 0.68	7.51 ± 0.81
Liver	Abs. (g)	23.05 ± 2.98	23.30 ± 3.16	24.35 ± 4.84	22.89 ± 2.19
	Rel. (%)	4.08 ± 0.54	3.83 ± 0.42	3.94 ± 0.65	4.15 ± 0.31
Testis	Abs. (g)	3.56 ± 0.23	3.64 ± 0.26	3.47 ± 0.20	3.44 ± 0.25
	Rel. (%x10)	6.30 ± 0.66	6.01 ± 0.61	5.66 ± 0.51	6.27 ± 0.72
Thyroid/parathyroid	Abs. (mg)	30 ± 5	33 ± 2	32 ± 5	31 ± 4
	Rel. (%x10^3)	5.33 ± 0.91	5.49 ± 0.50	5.26 ± 0.87	5.55 ± 0.87

F0 - Females					
Body weight (g)		310 ± 12	324 ± 25	325 ± 24	296 ± 25
Adrenal	Abs. (mg)	72 ± 10	77 ± 12	82 ± 11	83 ± 10.
	Rel. (%x10^3)	23.2 ± 3.4	23.6 ± 4.2	25.0 ± 3.0	28.3* ± 5.0
Heart	Abs. (g)	1.23 ± 0.10	1.34 ± 0.16	1.31 ± 0.12	1.16 ± 0.11
	Rel. (%x10)	3.99 ± 0.38	4.13 ± 0.48	4.02 ± 0.30	3.93 ± 0.33
Kidney	Abs. (g)	2.41 ± 0.18	2.59* ± 0.18	2.76** ± 0.3	3.09** ± 0.50
	Rel. (%x10)	7.78 ± 0.57	8.02 ± 0.48	8.47* ± 0.66	10.59** ± 2.73
Liver	Abs. (g)	13.55 ± 1.31	13.79 ± 1.45	15.00 ± 2.35	14.15 ± 1.71
	Rel. (%)	4.38 ± 0.45	4.26 ± 0.30	4.60 ± 0.53	4.82 ± 0.84
Ovary	Abs. (mg)	155 ± 13	174 ± 29	155 ± 45	150 ± 28
	Rel. (%x10^2)	5.02 ± 0.39	5.43 ± 1.15	4.76 ± 1.33	5.05 ± 0.61
Thyroid/parathyroid	Abs. (mg)	24 ± 5	28 ± 5	29 ± 10	28 ± 5
	Rel. (%x10^3)	7.87 ± 1.56	8.68 ± 1.17	8.95 ± 2.56	9.56 ± 1.02
F1b - Males					
Body weight (g)		70 ± 11	64 ± 5	66 ± 3	53** ± 9
Adrenal	Abs. (mg)	25 ± 4	22 ± 6	24 ± 2	23 ± 4
	Rel. (%x10^3)	37.1 ± 7.0	34.8 ± 8.7	35.8 ± 3.9	43.6 ± 6.2
Heart	Abs. (g)	0.42 ± 0.07	0.36 ± 0.04	0.41 ± 0.06	0.32 ± 0.08
	Rel. (%x10)	6.11 ± 0.75	5.57 ± 0.35	6.17 ± 0.66	6.13 ± 0.73
Kidney	Abs. (g)	0.91 ± 0.21	0.87 ± 0.06	0.87 ± 0.09	0.77 ± 0.15
	Rel. (%x10)	13.10 ± 1.98	13.62 ± 0.69	13.19 ± 0.90	14.60 ± 0.62
Liver	Abs. (g)	3.59 ± 0.67	3.54 ± 0.39	3.55 ± 0.22	2.90 ± 0.64
	Rel. (%)	5.16 ± 0.58	5.55 ± 0.30	5.37 ± 0.41	5.65 ± 0.51
Testis	Abs. (g)	0.46 ± 0.12	0.37 ± 0.08	0.41 ± 0.06	0.31 ± 0.07
	Rel. (%x10)	6.58 ± 1.23	5.79 ± 0.93	6.14 ± 0.62	5.09 ± 0.62
Thyroid/parathyroid	Abs. (mg)	10 ± 2	10 ± 2	10 ± 2	9 ± 1
	Rel. (%x10^3)	13.74 ± 2.44	15.07 ± 2.41	15.24 ± 4.31	17.35 ± 2.76
F1b - Females					
Body weight (g)		61 ± 4	61. ± 6	59 ± 3	49** ± 8
Adrenal	Abs. (mg)	23 ± 2	25 ± 3	23 ± 2	21 ± 5
	Rel. (%x10^3)	37.1 ± 1.4	41.9 ± 6.5	38.8 ± 3.9	43.5 ± 7.0
Heart	Abs. (g)	0.37 ± 0.03	0.37 ± 0.03	0.31* ± 0.04	0.33 ± 0.05
	Rel. (%x10)	6.07 ± 0.49	6.14 ± 0.41	5.23 ± 0.85	6.65 ± 0.80
Kidney	Abs. (g)	0.80 ± 0.11	0.82 ± 0.01	0.85 ± 0.09	0.72 ± 0.09
	Rel. (%x10)	13.01 ± 1.30	13.63 ± 1.11	14.39 ± 1.34	14.71 ± 2.44
Liver	Abs. (g)	3.32 ± 0.49	3.11 ± 0.29	3.25 ± 0.50	2.71 ± 0.62
	Rel. (%)	5.40 ± 0.65	5.13 ± 0.55	5.52 ± 0.74	5.44 ± 0.52
Ovary	Abs. (mg)	36 ± 7	37 ± 7	29 ± 4	33 ± 6
	Rel. (%x10^2)	5.81 ± 1.12	6.13 ± 1.01	4.95 ± 0.75	6.76 ± 0.53
Thyroid/parathyroid	Abs. (mg)	8 ± 1	11 ± 2	10 ± 1	10 ± 1
	Rel. (%x10^3)	13.43 ± 1.94	18.04* ± 3.19	16.38 ± 2.27	18.73* ± 3.20
F1 - Males					
Body weight (g)		698 ± 108	698 ± 89	694 ± 113	585* ± 73
Adrenal	Abs. (mg)	64 ± 13	64 ± 17	63 ± 13	57 ± 8
	Rel. (%x10^3)	9.2 ± 1.4	9.1 ± 1.3	9.3 ± 2.4	9.9 ± 1.6
Heart	Abs. (g)	1.56 ± 0.29	1.77 ± 0.13	1.84 ± 0.27	1.59* ± 0.19
	Rel. (%x10)	2.67 ± 0.15	2.55 ± 0.25	2.68 ± 0.39	2.73 ± 0.20
Kidney	Abs. (g)	4.41 ± 0.77	4.60 ± 0.36	4.55 ± 0.65	4.19 ± 0.42
	Rel. (%x10)	6.32 ± 0.43	6.64 ± 0.59	6.63 ± 0.88	7.21* ± 0.78
Liver	Abs. (g)	26.49 ± 5.07	23.85 ± 2.20	23.55 ± 3.28	20.85** ± 2.27
	Rel. (%)	3.78 ± 0.370	3.44 ± 0.305	3.43 ± 0.441	3.57 ± 0.180
Testis	Abs. (g)	3.06 ± 0.43	3.85 ± 0.33	3.54 ± 0.46	3.49 ± 0.26
	Rel. (%x10)	5.60 ± 0.82	5.57 ± 0.56	5.20 ± 1.03	6.02 ± 0.56
Thyroid/parathyroid	Abs. (mg)	35 ± 5	36 ± 6	37 ± 5	30 ± 7
	Rel. (%x10^3)	5.15 ± 0.93	5.23 ± 1.08	5.43 ± 1.39	6.48* ± 1.03
F1 - Females					
Body weight (g)		331 ± 29	355 ± 48	329 ± 42	315 ± 35
Adrenal	Abs. (mg)	69 ± 11	66 ± 6	64 ± 11	70 ± 13
	Rel. (%x10^3)	21.0 ± 4.2	18.9 ± 2.8	19.6 ± 2.8	22.4 ± 3.9

Heart	Abs. (g)	1.15 ± 0.08	1.17 ± 0.09	1.14 ± 0.10	1.19 ± 0.19
	Rel. (%x10)	3.51 ± 0.42	3.34 ± 0.35	3.48 ± 0.34	3.77 ± 0.46
Kidney	Abs. (g)	2.45 ± 0.19	2.53 ± 0.27	2.39 ± 0.27	2.63 ± 0.61
	Rel. (%x10)	7.46 ± 0.96	7.17 ± 0.65	7.33 ± 0.83	8.29 ± 1.34
Liver	Abs. (g)	12.75 ± 1.49	12.26 ± 1.75	11.23 ± 1.74	11.21 ± 2.04
	Rel. (%)	3.86 ± 0.46	3.46* ± 0.15	3.42* ± 0.37	3.54 ± 0.31
Ovary	Abs. (mg)	140 ± 28	132 ± 27	121 ± 24	142 ± 23
	Rel. (%x10^2)	4.23 ± 0.83	3.73 ± 0.69	3.70 ± 0.81	4.59 ± 1.11
Thyroid/parathyroid	Abs. (mg)	28 ± 6	30 ± 6	26 ± 7	29 ± 8
	Rel. (%x10^3)	8.39 ± 1.94	8.46 ± 1.11	7.97 ± 1.61	9.09 ± 2.30
F2b - Males					
Body weight (g)		54 ± 7	49 ± 6	50 ± 7	41 ± 8
Adrenal	Abs. (mg)	23 ± 7	20 ± 5	21 ± 4	18 ± 3
	Rel. (%x10^3)	42.8 ± 10.6	42.2 ± 10.6	41.5 ± 8.0	44.5 ± 4.9
Heart	Abs. (g)	0.31 ± 0.05	0.30 ± 0.06	0.30 ± 0.06	0.24 ± 0.04
	Rel. (%x10)	5.79 ± 0.59	6.17 ± 0.60	6.01 ± 0.48	6.55 ± 1.10
Kidney	Abs. (g)	0.70 ± 0.10	0.64 ± 0.10	0.65 ± 0.13	0.53 ± 0.10
	Rel. (%x10)	13.12 ± 0.57	13.35 ± 0.44	12.77 ± 0.90	13.63 ± 1.52
Liver	Abs. (g)	2.65 ± 0.38	2.26 ± 0.39	2.30 ± 0.44	1.74* ± 0.57
	Rel. (%)	4.95 ± 0.29	4.72 ± 0.36	4.56 ± 0.49	4.48 ± 0.54
Testis	Abs. (g)	0.29 ± 0.05	0.26 ± 0.04	0.27 ± 0.04	0.27 ± 0.12
	Rel. (%x10)	5.32 ± 0.58	5.42 ± 0.32	5.31 ± 0.42	7.57 ± 3.65
Thyroid/parathyroid	Abs. (mg)	7 ± 2	8 ± 1	8 ± 2	9 ± 2
	Rel. (%x10^3)	13.90 ± 3.67	17.25 ± 1.77	14.92 ± 2.68	21.44* ± 3.79
F2b - Females					
Body weight (g)		55 ± 11	47 ± 6	49 ± 6	39** ± 5
Adrenal	Abs. (mg)	20 ± 4	19 ± 4	20 ± 3	17 ± 5
	Rel. (%x10^3)	37.2 ± 6.1	40.9 ± 4.6	40.7 ± 5.5	44.5 ± 10.5
Heart	Abs. (g)	0.33 ± 0.06	0.29 ± 0.06	0.30 ± 0.05	0.24 ± 0.04
	Rel. (%x10)	6.03 ± 0.13	6.09 ± 0.94	6.02 ± 0.68	6.19 ± 0.47
Kidney	Abs. (g)	0.78 ± 0.18	0.68 ± 0.10	0.69 ± 0.09	0.54* ± 0.08
	Rel. (%x10)	14.14 ± 1.27	14.29 ± 0.10	13.89 ± 0.60	13.89 ± 0.93
Liver	Abs. (g)	2.66 ± 0.78	2.23 ± 0.37	2.43 ± 0.30	1.72* ± 0.34
	Rel. (%)	4.74 ± 0.45	4.70 ± 0.28	4.93 ± 0.21	4.40 ± 0.46
Ovary	Abs. (mg)	30 ± 6	31 ± 3	29 ± 11	29 ± 7
	Rel. (%x10^2)	5.48 ± 0.81	6.55 ± 0.93	5.81 ± 2.06	7.46 ± 1.64
Thyroid/parathyroid	Abs. (mg)	8 ± 3	8 ± 2	8 ± 1	7 ± 1
	Rel. (%x10^3)	13.80 ± 4.01	17.34 ± 4.11	15.93 ± 1.98	18.23 ± 2.65

* $p < 0.05$; ** $p < 0.01$

Incidence of microscopic observations in kidney

mg/kg bw/d / Sex		0		22.5		55.2		147	
Effect	**Grade**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
F0 (N = 10)									
Within normal limits	-	5	6	6	8	2	4	2	1
Pyelitis	Mild	1	0	0	0	2	0	0	0
Progressive nephropathy	Trace	2	0	0	1	2	3	3	3
	Mild	0	0	0	0	2	0	3	3
	Moderate	0	0	0	0	0	0	0	2
	Severe	0	0	0	0	0	0	0	1
Hydronephrosis	Mild	0	0	0	1	0	1	0	0
Inflammation	Mild	0	0	0	0	1	0	0	0
Tubular dilatation	Mild	0	0	0	0	0	0	1	3
	Moderate	0	0	0	0	0	0	1	2
Microconcretion	Trace	0	0	0	0	0	0	1	0
	Mild	0	0	0	0	1	0	1	0
Mineralization	Trace	0	1	1	0	0	2	0	0
	Mild	1	3	1	0	0	0	0	0
Lymphocytic infiltration	Trace	1	0	1	0	1	1	0	0
	Moderate	0	0	0	0	0	0	0	1
Hyaline cast	Trace	0	0	0	0	1	0	1	0

	Mild	2	0	2	1	1	0	1	0
F1b (N = 5)									
Within normal limits	-	3	3	5	2	4	5	4	5
Cyst	Trace	0	0	0	0	1	0	0	0
	Mild	0	0	0	2	0	0	0	0
Fibrosis	Mild	0	1	0	0	0	0	0	0
Hydronephrosis	Trace	0	0	0	1	0	0	0	0
	Mild	2	1	0	0	0	0	1	0
	Moderate	0	0	0	0	0	0	1	0
F1 (N = 10)									
Within normal limits	-	6	8	4	7	3	6	5	5
Calculus	Mild	0	1	0	0	0	0	0	0
	Moderate	0	0	0	0	1	0	0	0
Chronic progressive nephropathy	Moderate	0	0	0	0	0	0	1	1
Cyst	Mild	0	0	1	0	0	0	1	0
Hyaline cast	Trace	0	0	1	0	0	0	0	0
	Mild	0	1	1	1	2	3	2	0
Tubular degeneration	Trace	2	0	2	0	1	0	0	2
	Mild	1	0	1	0	2	0	1	0
Tubular dilatation	Mild	0	0	0	0	0	0	0	1
	Moderate	0	0	0	0	0	0	0	1
Hydronephrosis	Mild	0	0	0	0	0	1	0	0
	Moderate	0	1	0	0	1	0	0	0
Inflammation	Trace	0	0	0	1	0	0	0	0
	Mild	0	0	0	1	1	0	0	0
Lymphocytic infiltration	Trace	1	0	0	0	0	1	0	1
	Mild	0	0	0	0	0	0	1	1
Mineralization	Trace	0	1	0	0	0	0	0	0
	Mild	0	0	0	0	1	0	0	0
Pyelitis	Trace	0	1	0	0	0	0	0	0
F2b (N = 5)									
Within normal limits	-	4	3	4	5	5	5	5	5
Hyaline cast	Mild	1	0	0	0	0	0	0	0
Tubular dilatation	Mild	0	1	0	0	0	0	0	0
Hydronephrosis	Mild	0	1	1	0	0	0	0	0
Interstitial fibrosis	Mild	0	1	0	0	0	0	0	0

In the one-generation study (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.

Body weights values (g)

mg/kg bw/d / Week	0	20	40	0	20	40
Generation	**F0 males**			**F0 females**		
0	160	161	160	-	-	-
1	216	219	220	-	-	-
2	271	272	273	-	-	-
3	315	319	313	-	-	-

4	348	356	345	-		-	-
5	370	378	364	-		-	-
6	391	399	383	-		-	-
7	418	417	400		200	197	199
8	440	440	417		228	223	224
9	435	449	413		239	237	231
10	439	461	424	-		-	-
11	459	483	445	-		-	-
12	475	496	463	-		-	-
13	497	509	480	-		-	-
14	515	533	503	-		-	-
15	521	542	508	-		-	-
16	532	551	517	-		-	-
17	548	572	529	-		-	-
18	557	580	534		319	344	330
19	567	590	546		323	351	336

Body weights values (g)

mg/kg bw/d / Week	0	20	40	0	20	40	0	20	40	0	20	40
Generation	**F0 males**			**F0 females**			**F1 gestation**			**F1 post-partum**		
0	160	161	160	-	-	-	263	250	261	321	309	318
1	216	219	220	-	-	-	-	-	-	-	-	-
2	271	272	273	-	-	-	-	-	-	-	-	-
3	315	319	313	-	-	-	-	-	-	-	-	-
4	348	356	345	-	-	-	-	-	-	-	-	-
5	370	378	364	-	-	-	-	-	-	-	-	-
6	391	399	383	-	-	-	-	-	-	-	-	-
7	418	417	400	200	197	199	290	283	286	337	330	340
8	440	440	417	228	223	224	-	-	-	-	-	-
9	435	449	413	239	237	231	-	-	-	-	-	-
10	439	461	424	-	-	-	-	-	-	-	-	-
11	459	483	445	-	-	-	-	-	-	-	-	-
12	475	496	463	-	-	-	-	-	-	-	-	-
13	497	509	480	-	-	-	-	-	-	-	-	-
14	515	533	503	-	-	-	323	317	327	347	348	351
15	521	542	508	-	-	-	-	-	-	-	-	-
16	532	551	517	-	-	-	-	-	-	-	-	-
17	548	572	529	-	-	-	-	-	-	-	-	-
18	557	580	534	319	344	330	-	-	-	-	-	-
19	567	590	546	323	351	336	-	-	-	-	-	-
20	-	-	-	-	-	-	390	385	387	-	-	-
21	-	-	-	-	-	-	-	-	-	334	337	340

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, no classification and labelling for sexual function and fertility is required.

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

Conclusion used in Risk Assessment – Effects on fertility	
Value/conclusion	NOAEL for systemic toxicity (F0) = 10 mg/kg bw/day NOAEL for systemic toxicity (F1) = 50 mg/kg bw/day NOAEL for reproduction and fertility (F0, F1) = 50 mg/kg bw/day
Justification for the value/conclusion	Toxicity to reproduction was assessed in two 2-generation studies and a 1-generation study in the rat. Overt signs of systemic toxicity at the highest dose level were decreased body weight gain and food consumption, altered absolute and relative liver and kidney weights. No effects on the reproduction at dose levels lacking clear systemic toxicity were observed. The key values are based on the most reliable, most conservative 2-generation study available for Bronopol.

A.3.10.2 Developmental toxicity, Teratogenicity

Table 74: 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	Results, maternal/parental	Results, developmental	Remarks	Reference
OECD TG 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	NOAEL maternal toxicity = 10 mg/kg bw/day NOAEL Teratogenicity Embryotoxicity = 10 mg/kg	Dams: clinical signs, ↓ bw gain and food consumption	Fetuses: slightly ↑ malformations (related to maternal toxicity)		[redacted] and [redacted] 2006 (A6_08_1-1) [redacted]

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	Results, maternal/parental	Results, developmental	Remarks	Reference
		bw/day Exposure period: Day 7-27 of gestation	bw/day				
EPA OPP 83-3, similar to OECD TG 414, Oral (gavage), GLP Rel. 1 Supportive	Rabbit, New Zealand White, female, 18 to 20/group	Bronopol (99.8%) Doses: 0, 5, 20, 40, 80 mg/kg bw/day (based on a range-finding study) Exposure period: Day 7 to 19 of gestation Sacrifice on day 28 of gestation	NOAEL maternal toxicity = 40 mg/kg bw/day 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)	Maternal Mortality: No treatment-related mortalities Clinical symptoms of toxicity: In the high-dose group reduced size/quantity of fecal pellets, decreased food consumption, decrease in maternal body weight gain was observed. Necropsy: Inconspicuous	Teratogenicity/ Embryotoxicity: Overt signs of teratogenicity were observed 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 minor skeletal abnormalities (29.5% *vs*. 10.2% in the control group). Increased incidence of fetuses with unossified forelimb (8%) and hindlimb (16%) epiphyses.		[redacted] 1991 (A6.08.1_01 (reported in US EPA RED 1995)) Range finding study: [redacted] 1991 (A6.08.1_04) [redacted]
EPA OPP 83-3, similar to OECD TG 414, Oral (gavage), GLP Rel. 2 (stability was not provided) **Supportive**	Rat, Sprague-Dawley, mated females, 24/group	Bronopol (≥99.5%) Vehicle: acidified purified water, pH 4 Doses:	NOAEL maternal toxicity = 80 mg/kg bw/day NOAEL developmental	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	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 advanced ossification of the forelimb phalanges in both the 28 and the 80		[redacted] 1995 (A6.08.1_02 (reported in US EPA RED 1995)) [redacted]

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	Results, maternal/parental	Results, developmental	Remarks	Reference
		0, 10, 28, 80 mg/kg bw/day (based on a range-finding study) Exposure period: Day 6 to 15 of gestation Sacrifice on day 20 of gestation	toxicity = 80 mg/kg bw/day	back to control level. Necropsy: Inconspicuous	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 (no guideline) **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 (phase II) Exposure period: Day 6 to 15 of gestation Sacrifice on day 20 of gestation	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 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	No signs of developmental toxicity could be evidenced; in fact, all considered parameters were inconspicuous.		[redacted] 1993 (A6.08.1_05) [redacted]

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	Results, maternal/parental	Results, developmental	Remarks	Reference
				haemorrhaging of the stomach glandular mucosa and gas in the caecum, extensive ulceration within the glandular stomach mucosa and colon contents were dehydrated Necropsy of dams at end of study: Inconspicuous			
Non-guideline, Oral (gavage), Non-GLP Rel. 2 (no guideline, non-GLP) **Supportive**	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 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 of no statistical significance. Abnormalities in pups: None		[redacted] and [redacted] 1973 (A6.08.1_03) [redacted]

No human data addressing or 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 (A6.08.1_01, 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 (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 (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 (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 (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 (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.

BW gains (kg ± SD) – Pregnant Females

Dose (mg/kg bw/d)		Days of gestation								
		0-7	**7-8**	**7-9**	**9-12**	**12-15**	**15-19**	**7-19**	**19-28**	**0-28**
0	MEAN	0.10 ±0.10	0.02 ±0.03	0.05 ±0.06	0.06 ±0.04	0.09 ±0.10	0.04 ± 0.08	0.23 ±0.11	0.20 ±0.12	0.02 ±0.20
	N =	18	18	18	18	18	18	18	18	18
5	MEAN	0.11 ±0.11	0.03 ±0.03	0.06 ±0.04	0.06 ±0.06	0.13 ±0.07	0.02 ±0.11	0.28 ±0.16	0.23 ±0.12	0.09 ±0.14
	N =	17	17	17	17	17	17	17	17	17
20	MEAN	0.01 ±0.10*	0.03 ±0.03	0.06 ±0.06	0.06 ±0.05	0.08 ± 0.06	0.07 ±0.08	0.27 ±0.10	0.22 ±0.11	-0.02 ±0.21
	N =	19	19	19	19	19	19	19	19	19
40	MEAN	0.13 ±0.13	0.02 ±0.04***	0.03 ±0.06***	0.09 ±0.06	0.12 ± 0.05	0.04 ±0.08	0.27 ±0.08*	0.23 ±0.06	0.11 ±0.16
	N =	19	19	19	19	19	19	19	19	19
80	MEAN	0.14 ±0.15	-0.06 ±0.06	-0.06 ±0.11	0.02 ±0.17	0.12 ±0.21	0.08 ±0.23	0.05 ±0.14	0.27 ±0.11	0.04 ±0.23
	N =	19	19	19	19	19	19	19	19	19

*p < 0.05; ***p < 0.001; Student's t test

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12	

Maternal food consumption (g/rabbit/d)

Dose (mg/kg bw/d)		Days of gestation					
		3-7	7-11	11-15	15-19	19-23	23-28
0	MEAN	167±36	181±31	147±46	150±29	167±39	136±30
	N =	18	18	18	18	18	18
5	MEAN	163±42	187±33	163±50	170±52	173±39	130±31
	N =	17	17	17	17	17	17
20	MEAN	148±48	181±40	154±40	161±52	177±41	144±45
	N =	18	19	19	19	18	19
40	MEAN	164±41	180±48	163±40	169±42	173±28	156±31
	N =	18	19	19	19	19	19
80	MEAN	174±50	113±78***	119±163	145±59	185±26	162±29*
	N =	17	19	19	19	19	19

*p < 0.05, Student's t test; *** p < 0.001, Student's t test; () = entries different from stated N

Foetal weight (g±S.D)

Dose (mg/kg bw/d)	M	F	M+F
0	36.0±6.0 (18)	35.6±5.6 (18)	35.8±5.6 (18)
5	35.3±4.1 (17)	35.6±4.8 (17)	35.5±4.4 (17)
20	36.7±5.0 (18)	36.4±5.0 (19)	36.8±5.0 (19)
40	34.6±3.8 (19)	34.5±4.5 (19)	34.6±3.9 (19)
80	32.9±5.3 (18)	31.7±5.1* (19)	32.1±5.0* (19)

*p<0.05, Student's t test; () = entries different from stated N

Foetal examination

Parameter \ mg/kg bw/d	0	5	20	40	80
Litters	18	17	19	19	19
Foetuses	159	165	178	182	193
External visceral examination					
Minor abnormalities only (%)	38 (23.9)	36 (21.8)	23 (12.9)	29 (15.9)	30 (15.5)
Major abnormalities (%)	0 (0.0)	3 (1.8)	4 (2.3)	1 (0.6)	6 (3.1)
Skeletal examination					
Minor abnormalities only (%)	18 (11.3)	22 (13.3)	10 (5.6)	27 (14.8)	60 (31.1)**
Major abnormalities (%)	0 (0.0)	2 (1.2)	4 (2.3)	1 (0.6)	7 (3.6)
Combined examinations					
Any major abnormality (%)	0 (0.0)	7 (4.2)	4 (2.3)	2 (1.1)	12 (6.2)
Litters with foetuses with major abnormalities	0 (0.0)	4 (23.5)	6 (31.6)	2 (10.5)	6 (31.6)

** p<0.01, Dunn's multiple comparison test

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) (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.

BW gains (g) – Pregnant Females

Dose (mg/kg bw/d)		Days of gestation								
		0-7	7-10	10-13	13-16	16-20	20-24	24-28	7-28	0-28
0	MEAN	100.5	23.4	42.3	70.4	74.6	51.1	48.5	310.3	410.8
	S.D.	86.9	35.8	37.6	50.3	38.0	42.6	59.2	110.8	156.4
	N =	25	25	25	25	25	25	25	25	25
3	MEAN	121.7	20.3	58.1	67.5	56.7	59.9	50.8	313.3	435.0

	S.D.	91.6	37.3	31.7	46.3	45.2	29.6	58.0	70.2	109.2
	N =	24	24	24	24	24	24	24	24	24
	MEAN	125.0	-2.5	62.4	79.6	47.8	64.5	57.9	320.3	444.3
10	S.D.	89.2	57.6	66.7	59.7	74.7	42.4	63.7	133.1	181.4
	N =	26	26	26	26	26	25*	24*	24	24
	MEAN	123.0	-40.2	43.0	68.9	46.9	72.0	44.9	249.2	364.1
30	S.D.	95.0	111.0	97.9	97.6	98.0	76.3	72.0	144.9	190.9
	N =	26	26	26	26	26	25*	25	25	25

*Varying (N) values due to death of animals 8042 (10 mg/kg bw/day - Day 23), 8043 (10 mg/kg bw/day - Day 26), and 8059 (30 mg/kg bw/day - Day 23).

Clinical Observations

Dose (mg/kg bw/d)		0	3	10	30
N =		26	26	26	26
All categories	Within normal limits	11	14	12	8
Feces	Abnormal quantity, absent	0	0	1	1
	Abnormal quantity, decreased	7	5	6	15
	Abnormal quantity, soft	0	1	0	0
Injury	Apparent mechanical, other	2	0	1	0
	Apparent mechanical, laceration(s)	0	0	1	0
	Apparent mechanical, scratch(es)	8	5	7	4
Miscellaneous	Blood in cage	1	1	0	0
	Cold to touch	0	0	0	1
Posture	Head tilt	0	0	1	1
Reproductive system	Vulvar discharge, red	0	1	0	0
	Vulva enlarged	0	0	1	0
Respiration	Slow	0	0	2	0
	Noisy	0	0	0	5
	Labored, without mouth-breathing	0	0	0	1
Skin/fur/mucous membranes	Ungroomed appearance	0	1	0	0
	Skin/mucous membranes pale	0	1	0	0
	Excessive hairloss	1	0	0	0
Soiling	Perioral, clear	0	0	3	1
	Perineal,urine	0	0	1	0
	Perineal, urine & fecal	0	1	0	0
	Perinasal, clear	0	0	4	1
Disposition	Moribund - unscheduled	0	0	1	1
	Scheduled necropsy	26	26	24	25
	Spontaneous - unscheduled	0	0	1	0

Incidence of Fetal Alterations

Dose (mg/kg bw/d)			0	3	10	30
			Number affected/total number (% affected)			
External observations	Subdermal hematoma general	F	1/224 (0.4)	0/200 (0.0)	0/209 (0.0)	0/215 (0.0)
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Hernia umbilicus	F	1/224 (0.4)	0/200 (0.0)	0/209 (0.0)	0/215 (0.0)
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Filamentous tail+	F	0/224 (0.0)	0/200 (0.0)	0/209 (0.0)	1/215 (0.5)[a]
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Hypoplastic tail+	F	0/224 (0.0)	0/200 (0.0)	1/209 (0.5)	0/215 (0.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
Craniofacial observations		F	No observations			
		L				
Visceral observations	Paraovarian cyst ovary	F	3/116 (2.6)	2/107 (1.9)	1/108 (0.9)	1/110 (0.9)
		L	3/25 (12.0)	2/24 (8.3)	1/24 (4.2)	1/25 (4.0)
	Paratesticular cyst testis	F	2/108 (1.9)	0/93 (0.0)	0/101 (0.0)	0/105 (0.0)
		L	2/25 (8.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Right-sided esophagus	F	0/224 (0.0)	2/200 (1.0)	0/209 (0.0)	7/215 (3.3)
		L	0/25 (0.0)	2/24 (8.3)	0/24 (0.0)	3/25 (12.0)

	Hemorrhage thymus	F	2/224 (0.9)	9/200 (4.5)	3/209 (1.4)	0/215 (0.0)
		L	2/25 (8.0)	6/24 (25.0)	3/24 (12.5)	0/25 (0.0)
	Fused lung	F	2/224 (0.9)	0/200 (0.0)	1/209 (0.5)	0/215 (0.0)
		L	2/25 (8.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
	Missing caudal lung	F	13/224 (5.8)	1/200 (0.5)*	5/209 (2.4)	17/215 (7.9)
		L	9/25 (36.0)	1/24 (4.2)	5/24 (20.8)	11/25 (44.0)
	Hemorrhage liver, median lobe	F	1/224 (0.4)	0/200 (0.0)	0/209 (0.0)	0/215 (0.0)
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Torsion strangulation liver, median lobe	F	0/224 (0.0)	1/200 (0.5)	0/209 (0.0)	1/215 (0.5)
		L	0/25 (0.0)	1/24 (4.2)	0/24 (0.0)	1/25 (4.0)
	Missing gall bladder+	F	0/224 (0.0)	1/200 (0.5)	0/209 (0.0)	0/215 (0.0)
		L	0/25 (0.0)	1/24 (4.2)	0/24 (0.0)	0/25 (0.0)
	Hypoplastic spleen	F	0/224 (0.0)	0/200 (0.0)	1/209 (0.5)	0/215 (0.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
	Hypoplastic pancreas	F	0/224 (0.0)	0/200 (0.0)	1/209 (0.5)	0/215 (0.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
	Ectopic kidney+	F	1/224 (0.4)[b]	0/200 (0.0)	0/209 (0.0)	3/215 (1.4)[c,d,e]
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Hypoplastic kidney	F	1/224 (0.4)	0/200 (0.0)	0/209 (0.0)	0/215 (0.0)
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Fused kidney+	F	1/224 (0.4)[b]	0/200 (0.0)	0/209 (0.0)	1/215 (0.5)[c]
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Hydroureter ureter+	F	1/224 (0.4)[b]	0/200 (0.0)	0/209 (0.0)	3/215 (1.4)[c,d,e]
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Retrocaval ureter	F	4/224 (1.8)	0/200 (0.0)	2/209 (1.0)	3/215 (1.4)
		L	3/25 (12.0)	0/24 (0.0)	2/24 (8.3)	2/25 (8.0)
	Convoluted ureter	F	0/224 (0.0)	1/200 (0.5)	0/209 (0.0)	0/215 (0.0)
		L	0/25 (0.0)	1/24 (4.2)	0/24 (0.0)	0/25 (0.0)
	Hypoplastic heart+	F	0/224 (0.0)	0/200 (0.0)	1/209 (0.5)	0/215 (0.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
Skeletal observations	Delayed ossification interparietal	F	0/107 (0.0)	0/93 (0.0)	1/98 (1.0)	1/99 (1.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	1/25 (4.0)
	Delayed ossification hyoid	F	42/107 (39.3)	36/93 (38.7)	41/98 (41.8)	38/99 (38.4)
		L	18/25 (72.0)	17/24 (70.8)	17/24 (70.8)	18/25 (72.0)
	Crooked hyoid	F	2/107 (1.9)	2/93 (2.2)	3/98 (3.1)	7/99 (7.1)
		L	2/25 (8.0)	2/24 (8.3)	3/24 (12.5)	6/25 (24.0)
	Delayed ossification dentoid	F	0/107 (0.0)	0/93 (0.0)	0/98 (0.0)	1/99 (1.0)
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Extra site of ossification dentoid	F	2/107 (1.9)	0/93 (0.0)	0/98 (0.0)	0/99 (0.0)
		L	2/25 (8.0)	0/24 (0.0)	0/24 (0.0)	0/25 (0.0)
	Delayed ossification cervical centra	F	0/107 (0.0)	0/93 (0.0)	1/98 (1.0)	0/99 (0.0)
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	0/25 (0.0)
	Fused cervical centra+	F	0/107 (0.0)	0/93 (0.0)	0/98 (0.0)	1/100 (1.0)[#]
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Delayed ossification thoracic centra	F	0/224 (0.0)	1/200 (0.5)	0/209 (0.0)	2/215 (0.9)
		L	0/25 (0.0)	1/24 (4.2)	0/24 (0.0)	1/25 (4.0)
	Fused thoracic rib+	F	0/224 (0.0)	1/200 (0.5)	0/209 (0.0)	1/215 (0.5)[f]
		L	0/25 (0.0)	1/24 (4.2)	0/24 (0.0)	1/25 (4.0)
	Fused thoracic centra+	F	0/224 (0.0)	0/200 (0.0)	0/209 (0.0)	2/215 (0.9)[a,f]
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Extra thoracic rib+	F	0/224 (0.0)	0/200 (0.0)	0/209 (0.0)	1/215 (0.5)[f]
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Hemivertebra thoracic vertebrae+	F	1/224 (0.4)	0/200 (0.0)	0/209 (0.0)	2/215 (0.9)[a,f]
		L	1/25 (4.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Forked ribs+	F	0/224 (0.0)	0/200 (0.0)	1/209 (0.5)	1/215 (0.5)[a]
		L	0/25 (0.0)	0/24 (0.0)	1/24 (4.2)	1/25 (4.0)
	Delayed ossification ribs	F	0/224 (0.0)	0/200 (0.0)	0/209 (0.0)	1/215 (0.5)
		L	0/25 (0.0)	0/24 (0.0)	0/24 (0.0)	1/25 (4.0)
	Delayed ossification sternebrae	F	104/224 (46.4)	72/200 (36.0)	72/209 (34.4)	78/215 (36.3)
		L	22/25 (88.0)	19/24 (79.2)	20/24 (83.3)	23/25 (92.0)

	Fused sternebrae	F L	3/224 (1.3) 3/25 (12.0)	1/200 (0.5) 1/24 (4.2)	0/209 (0.0) 0/24 (0.0)	2/215 (0.9) 2/25 (8.0)
	Extra site of ossification sternebrae	F L	1/224 (0.4) 1/25 (4.0)	1/200 (0.5) 1/24 (4.2)	1/209 (0.5) 1/24 (4.2)	0/215 (0.0) 0/25 (0.0)
	Irregular pattern of ossification sternebrae	F L	3/224 (1.3) 3/25 (12.0)	3/200 (1.5) 3/24 (12.5)	1/209 (0.5) 1/24 (4.2)	0/215 (0.0) 0/25 (0.0)
	Extra lumbar rib 2 through 6[+]	F L	2/224 (0.9)[b] 2/25 (8.0)	0/200 (0.0) 0/24 (0.0)	1/209 (0.5) 1/24 (4.2)	4/215 (1.9) 2/25 (8.0)
	Delayed ossification pubis	F L	8/224 (3.6) 4/25 (16.0)	7/200 (3.5) 7/24 (29.2)	7/209 (3.3) 5/24 (20.8)	16/215 (7.4) 5/25 (20.0)
	Delayed ossification talus	F L	2/224 (0.9) 2/25 (8.0)	1/200 (0.5) 1/24 (4.2)	2/209 (1.0) 2/24 (8.3)	3/215 (1.4) 2/25 (8.0)
Total malformed[@]	Total malformed external	F L	0/224(0.0) 0/25(0.0)	0/200(0.0) 0/24(0.0)	1/209(0.5) 1/24(4.2)	1/215(0.5) 1/25(4.0)
	Total malformed craniofacial	F L	0/117(0.0) 0/25(0.0)	0/107(0.0) 0/24(0.0)	0/111(0.0) 0/24(0.0)	0/116(0.0) 0/25(0.0)
	Total malformed visceral	F L	1/224(0.4) 1/25(4.0)	1/200(0.5) 1/24(4.2)	1/209(0.5) 1/24(4.2)	3/215(1.4) 1/25(4.0)
	Total malformed visceral (male)	F L	0/108(0.0) 0/25(0.0)	0/93(0.0) 0/24(0.0)	0/101(0.0) 0/23(0.0)	0/105(0.0) 0/25(0.0)
	Total malformed visceral (female)	F L	0/116(0.0) 0/25(0.0)	0/107(0.0) 0/24(0.0)	0/108(0.0) 0/23(0.0)	0/110(0.0) 0/24(0.0)
	Total malformed skeletal	F L	3/224(1.3) 3/25(12.0)	1/200(0.5) 1/24(4.2)	2/209(1.0) 2/24(8.3)	6/215(2.8) 3/25(12.0)
	Total malformed skeletal (head)	F L	0/107(0.0) 0/25(0.0)	0/93(0.0) 0/24(0.0)	0/98(0.0) 0/24(0.0)	1/100 (1.0)[#] 1/25 (4.0)

F = fetuses; L = litters.
[+]Considered a malformation.
[@]Not statistically analyzed.
[a-f]Malformations denoted with the same superscript were noted in a single fetus.
[#]One extra examination done on animal 8063. See note of clarification.
*Statistically different from control mean by censored Wilcoxin's test, α = 0.05.

This is in agreement with the findings of the rabbit developmental toxicity study conducted by 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 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, no classification and labelling for developmental toxicity is required.

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

Conclusion used in Risk Assessment – Effects on development	
Value/conclusion	NOAEL for systemic toxicity = 10 mg/kg bw/day NOAEL for embryotoxicity = 10 mg/kg bw/day
Justification for the value/conclusion	Developmental toxicity of Bronopol was assessed in the rat and in rabbits. Pups from rabbits treated with a Bronopol dose causing maternal toxicity (including decreased body weight gain and food consumption) showed some effects clearly linked to maternal toxicity (reduced foetal body weight and incidental abnormalities of general retardation of skeletal ossification and growth), whereas rat pups were unaffected up to the maximum tolerable dose level. The key values are based on the most reliable, most conservative developmental toxicity study available for rabbits exposed to Bronopol.

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. 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, no classification and labelling for reproductive toxicity is required.

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

Conclusion used in Risk Assessment – Reproductive toxicity	
Value	Bronopol is not a reproductive or developmental toxicant based on available study results. NOAEL for reproduction and fertility (F0, F1) = 50 mg/kg bw/day NOAEL for embryotoxicity = 10 mg/kg bw/day
Justification for the selected value	Based on the results of developmental and fertility studies, exposure to Bronopol did not affect fertility, reproductive performance or developing foetus in the pre- and in postnatal period of treated animals in the absence of severe systemic toxicity.
Proposed classification	No classification proposed.

A.3.11 Aspiration hazard

Bronopol is neither a liquid nor a hydrocarbon, it is a solid. Moreover, 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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.3.15 Further Human data

No further human data are available for Bronopol.

A.3.16 Other data

Table 75: 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% ([redacted], [redacted])) 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, b) [redacted]
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] *et al.* 1973 (A6.10_02) [redacted]

Conclusion used in Risk Assessment – Other data	
Conclusion	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 was determined. 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 hours of incubation, then the concentration of formaldehyde decreased slightly over time. Also, it was shown that Bronopol did not significantly induce methaemoglobin formation (methaemoglobinaemia) in minimal-disease cats at toxic dose levels.
Justification for the conclusion	Based on the mechanistic studies conducted with Bronopol.

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.

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. As formaldehyde has been evaluated under the Biocides Directive, please refer to the formaldehyde core dossier for specific information.

Regarding bromide ion, this can also be considered as a possible formation product. Nevertheless, the mode of action for Bronopol is not based on this ion formation, and hence it cannot be considered as a Bromide releaser. Regarding the possible background levels of bromide, an estimation is included in appendix III.

For deriving the degradation rates or the half-lives values at standard environmental temperatures, TAB ENV 182 should be applied, hence the Arrhenius equation in the form below to correct biodegradation rates in the temperature range of 0 to 30°C should be used (temperatures should be entered in degrees Kelvin):

$$k_{T_1} = k_{T_2} e^{\left(\frac{E_a}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right]\right)}$$

$$DT50_{T_1} = DT50_{T_2} e^{\left(\frac{E_a}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right]\right)}$$

Ea (activation energy) [J mol-1] = 65400 (54000 should be used for abiotic processes)

R (gas constant) [J mol-1 K-1] = 8.31447

A.4.1.1 Degradation

A.4.1.1.1 Abiotic degradation

Hydrolysis – Bronopol (parent compound)

Table 76: 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 TG 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	-	70[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_1-02)
	4	70	No data	1.1	0.998	7.539×10^{-6}	
	4	80	No data	0.26	0.999	3.105×10^{-5}	
	7	50	No data	< 0.7 h[b]	-		
	9	50	No data	< 0.1 h[b]	-	-	
OECD TG 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 (considering activation molar energy of 54 KJ/mol for abiotic process)

[b] Half-life estimated from preliminary test

In the first (key) study ([redacted] 2003), 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 50 ºC 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 70 days was estimated for 25°C (approximately 190 days at 12 ºC) by using Arrhenius equation from TAB ENV 182 with an Ea = 54 KJ/mol for abiotic processes, indicating that Bronopol is stable to hydrolysis under acidic conditions (pH 4). The half-life at pH 7 and 12°C was around 1.2 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; nevertheless, the results are in line with all the literature available and hence the conversion considered as valid). 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** (2-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 2-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 2-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 2-Hydroxymethyl-2-nitro-1,3-propanediol (trade name Tris(hydroxymethyl)nitromethane) and Formaldehyde. Other metabolites appeared below 10%.

The second hydrolysis study ([redacted] 2000) is not well documented. The tier 2 has been also performed at pH 4 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).

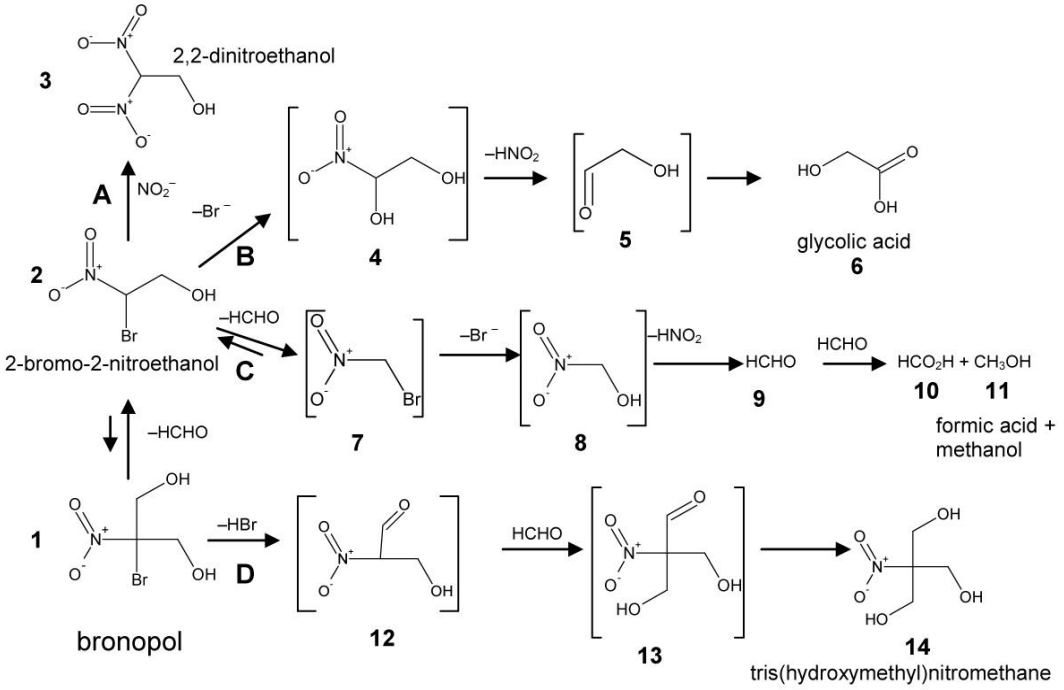
In the third (key, non-GLP) study ([redacted] 1996), hydrolysis of Bronopol was investigated according to OECD TG 111 (tier 1 test) at pH 4, 7 and 9 at 50°C in sterile buffer solutions. Rapid hydrolysis of Bronopol was both pH and concentration dependent. At the end of the test after 5 to 7 days, Bronopol 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, such as PT11 and 12. 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.

Considering the two key studies, both leading to a DT50 below 2 hours, it can be considered that the value of 0.0245 days (35.28 minutes) from [redacted]'study can be used for derivation of the degradation constant. This means that a k = 0.15 h^{-1} is selected, at 20 ºC (T for indoor uses such as PT11 or PT12).

In addition to those standard tests, a publication is available by [redacted] (1991). 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) 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 **2-Hydroxymethyl-2-nitro-1,3-propanediol (Tris(hydroxymethyl)nitromethane** or 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 2-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 2-BNE remained constant in equilibrium with the parent compound Bronopol.



Main degradation pathways described by [redacted] (1991). 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 2-Hydroxymethyl-2-nitro-1,3-propanediol (Tris(hydroxymethyl)nitromethane) (14).

Aside from the above-mentioned substances, **bromonitromethane** (BNM) additionally occurs in literature as degradation product of Bronopol ([redacted] 2011, [redacted] 2002, [redacted] 2000). According to [redacted] (2002), 2-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 2-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 [redacted] (2000) where 2-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 2-BNE.

Additionally, [redacted] *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 (2-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 [redacted] and [redacted] Even in the industrial products ([redacted] *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 2-BNE can also react with formaldehyde to produce the parent again. These results indicate that 2-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. [redacted] *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 bronopol. This supports the results from the studies presented, which is around ½ hour at 50 ºC and pH 7 and around 0.3 days at natural water temperature.

According to [redacted] (2000), the whole degradation process of Bronopol can be summed up as follows: Bronopol is degraded to 2-BNE by losing a formaldehyde molecule and 2-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 2-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). [redacted] (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. [redacted] (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.

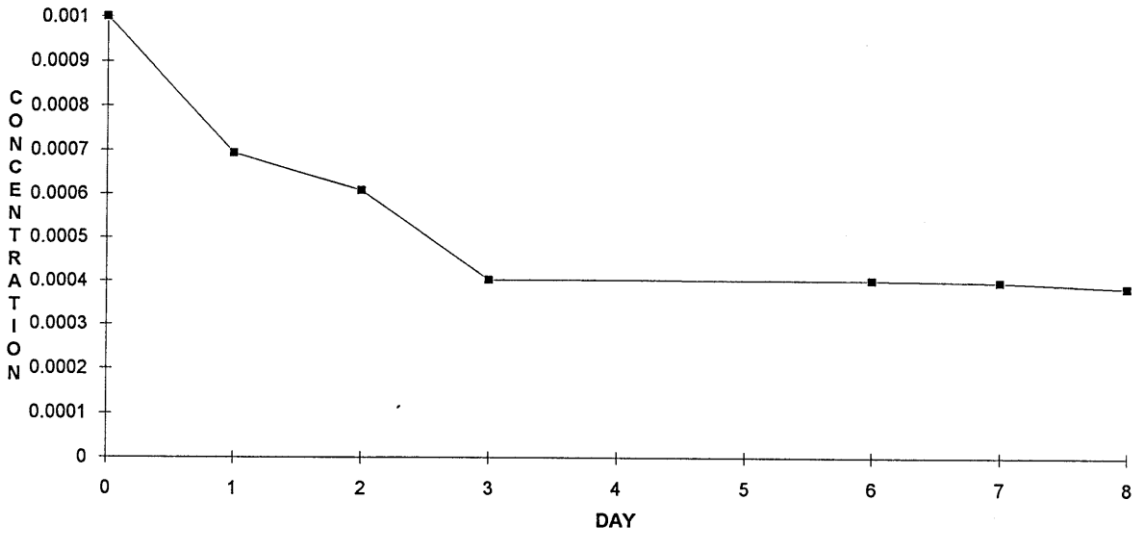
Hydrolysis – 2-hydroxymethyl-2-nitro-1,3-propanediol (trade name tris(hydroxymethyl)nitromethane) and 2-bromo-2-nitroethanol (metabolites)

Table 77: 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, equivalent to 9.2 days at 12ºC	No data	Degradation of TNM by Hydrolysis	[redacted] H. 1993 (A7.1.1.1.1_04)
OECD TG 111, GLP, Rel. 2	4	50	$3.0*10^{-5}$	stable	Not applicable for Tier 1 test	Degradation of 2-BNE	[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	-	

According tu [redacted], TNM 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 at 25ºC. Only formaldehyde was detected as hydrolysis product >10%. However, the presence of formaldehyde in closed vials was shown to stabilize TNM. This was expected based on the fact that TNM is synthesized by a reversible reaction of three moles of formaldehyde with one mole of nitromethane. This can be appreciated in the biphasic degradation behaviour of TNM degradation:



2-bromo-2-nitroethanol exhibited increasing hydrolysis rates with increasing pH. After 5 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 12 ºC and pH 7 was extrapolated from DT50 at 50 ºC using Arrhenius equation (TAB ENV 182, considering E_a = 54 KJ/mol for an abiotic process instead of 65.4 used for biotic degradation) leading to a value of around 13 days. This value is just an approximation, as the scope of the equation in TAB ENV 182 is from 0 to 30 ºC. Nevertheless, data from literature shows a hydrolysis DT50 of around 11-12 d in natural waters at pH 7 ([redacted] *et al.* 2010), which fits with the test results (0.91 days at 50 ºC = 7 days at 20 ºC = 13 days at 12 ºC). According to [redacted] *et al.*, 2-BNE as primary degradation product rapidly degraded to BNM in two water samples at neutral pH, whereas in other three water samples it decomposed at slower rates to produce BNM, but gave higher amounts of 2-BE via another degradation pathway. So hydrolysis seems not to be favoured in this case but another degradation pathway is. Hence, the value which seemed to be more related to hydrolysis at neutral pH is around 11-12 days. This shows that 2-BNE is more stable than Bronopol.

Compared to bronopol, degraded within a few hours at 25 ºC, the hydrolytic half-life of 2-BNE in natural waters is relatively high ([redacted] *et al.* 2010). The half-life varied due to the different pH values and water samples. 2-Bromo-2-nitroethanol (2-BNE) was catalytically degraded by natural waters, though this metabolite is hydrolytically more stable than parent ([redacted] *et al.* 2010).

Value used in Risk Assessment	
Value/conclusion	0.0245 d (DT_{50}, 50°C) 0.0465 d (DT_{50}, 40°C) 0.192 d (DT_{50}, 20°C) 0.357 d (DT_{50}, 12°C)
Justification for the value/ conclusion	At environmental relevant conditions (pH 7), the half-life time DT_{50} of Bronopol was only determined at 50°C. Since the DT_{50} is used in the environmental risk assessment calculations, the result was temperature-corrected for the average EU outdoor temperature of 12°C (for freshwater) and the standard temperature in cooling waters and paper mills according to TAB ENV 190 (20°C). Also 40 ºC was considered for the white water circuit in paper mills. The temperature normalisation from 50 ºC to 40, 20 and 12 ºC for the hydrolysis has been done by the Arrhenius equation in TAB ENV 182, although this is only applicable in principle from 0°C to 30°C. The results are anyway relevant compared and supported by literature from [redacted] *et al.* 2010, where hydrolysis was performed at 20 ºC.

Value used in Risk Assessment for degradation products	
Value/conclusion	TNM: 9.2 d (DT_{50}, 12°C) 2-BNE: 13 d (DT_{50}, 12°C)
Justification for the value/ conclusion	Both values are derived from literature. 2-BNE is considered an intermediate product. It reaches 44.2% (tier 2 test, pH 4). In tier 1, at pH 9, the concentration of 2-BNE diminishes after 24 hours. In tier 1, product A (could be a mixture of 2-Hydroxymethyl-2-nitro-1,3-propanediol and Formaldehyde) ranges 18.2% (pH 4) – 77.3% (pH 9).

Phototransformation in water

Table 78: 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). 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) 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 TNM and carbon dioxide. TNM 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.

Value used in Risk Assessment	
Value/conclusion	20 days (DT_{50}, 25°C)
Justification for the value/ conclusion	Value not used in risk assessment as it is not relevant for Bronopol.

Estimated photo-oxidation in air

Table 79: 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[6])	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-BNE 0.957E-012 TNM 1.923E-012 Formaldehyde 8.13E-012	2-BNE Half-life: 16.8 days. TNM 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.

[6] 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.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

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). Using a 24-hours day and a mean daily OH concentration in air of $1.5*10^6$ OH-radicals per cm^3, a half-life in air of 12.106 days was assessed (overall OH rate constant: 1.33 10^{-12} cm^3/molecule*sec). Based on these estimations, Bronopol will be rather slowly degraded in air.

Value used in Risk Assessment	
Value/conclusion	Half-life of 24 days (12 hrs sunlight)
Justification for the value/ conclusion	Value not used in risk assessment as it is not relevant for Bronopol.

A.4.1.1.2 Biotic degradation, initial studies

Biodegradability (ready/inherent)

Table 80: 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 TG 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 TG 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.	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%	Positive control (sodium benzoate): 98% degradation after	[redacted] 2001 (A7_1_1_2_1-02)

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 [%]		
to OECD TG 301E, GLP, Rel. 3 (supporting information)									within 7 days) not caused by microorganisms	14 days	
OECD TG 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 TG 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	67.24% at test end	Positive control (D[U-^{14}C]-Glucose): 67% degradation after 14 days	[redacted] 1994 (A7.1.1.2.2_01)
Studies on metabolites											
OECD TG 301F (1996), GLP, Rel. 2	Ready, 2-Hydroxymethyl-2-nitro-1,3-propanediol (TNM)	O_2 consumption	Activated sludge	30 mg/L (suspended solids)	no	no	19 and 46 mg/L TNM	28 days	13.4 at test end in 46 mg/L treatment, 8.9 at test end in 10 mg/L treatment Abiotic control: no degradation based on O_2 consumption	Positive control (sodium benzoate): >60% degradation after 3.3 days Inhibitory effects observed in toxicity control, although criteria for inhibition by test substance was not met (73% O_2	[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 [%]		
									and CO_2 production	consumption instead of <25% after 14 days)	
OECD TG 301F (1992), Com. Reg. (EC) No 440/2008 Method C.4-D, GLP, Rel. 4	Ready, 2-bromo-2-nitroethanol (2-BNE)	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 (2-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 TG 301B (1993), non-GLP, Rel. 3	Ready, 2-Bromo-2-nitroethanol (2-BNE)	$^{14}CO_2$ 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 TG 301B test ([redacted], 2002a), 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). ^{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 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 [redacted], 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). 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 [redacted] (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 TG 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). 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 TG 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), the use of radiolabelled active substance allowed the characterisation of 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), the biodegradation potential of TNM was directly evaluated using the manometric respirometry test (OECD TG 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** (2-BNE) as second major degradation product was also investigated in a study according to OECD TG 301F ([redacted] 2012). 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 2-BNE was conducted ([redacted] 2021). In this screening test based on OECD TG 301B ^{14}C-2-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 CO2. 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 10d-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 (14CO2) 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). 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.

Value used in Risk Assessment	
Value/conclusion	Bronopol: not readily biodegradable Main metabolites 2-BNE: readily biodegradable (only supporting information) TNM: not readily biodegradable
Justification for the value/ conclusion	In the studies on Bronopol, the threshold value given in the OECD guideline was not reached due to fast abiotic degradation processes like hydrolysis. The study on TNM showed signs on inhibition of the inoculum, this should be further investigated, *e.g.* by testing lower concentrations of TNM like it was done for 2-BNE, where the screening test confirmed the readily biodegradability of 2-BNE, although this test is only considered as supporting information. Studies with inhibitory test substance concentrations were not considered in evaluation.

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 2-BNE as reactive intermediate. It could be shown that at pH 9 and 25°C, 2-BNE is formed from Bronopol and increases in concentration over about 30 min following pseudo first-order kinetics. Thereafter, the concentration of 2-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 24 days (12 h sunlight) was calculated for this substance.

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

Aerobic biodegradation

Table 81: Summary table - STP aerobic biodegradation*

Method, Guideline, GLP status, Reliability, Key/supportive study	Test type[1]	Test parameter	Inoculum			Additional substrate	Test sub-stance concen-tration	Degradation		Remarks	Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]		
OECD TG 314B, GLP, Rel. 2	Primary and ultimate	Mineralisation, $^{14}CO_2$ evolution	Activated sludge from municipal STP	2500 mg suspended solids per litre	None	No	0.5 mg/L ^{14}C Bronopol	28 days	99 within first hour, but disappearance was not caused by micro-organisms; Cumulative $^{14}CO_2$ evolution: 66.8% AR by day 7, 77.8% AR after 28 days	Positive control (aniline): 64.8% degradation after 28 days; only one sludge was used.	[redacted] 2012 (A7_1_2_1_1)

Studies on metabolites											
OECD TG 314B, GLP, Rel. 1	Primary and ultimate	Mineralisation, $^{14}CO_2$ evolution for 2-bromo-2-nitroethanol (2-BNE)	Activated sludge from municipal STP	4000 mg dry substance per litre	none	no	0.6 mg/L ^{14}C 2-bromo-2-nitroethanol (2-BNE)	28 days	Rapid decline of 2-BNE, after 15 min no ^{14}C-2-BNE remaining in test solutions; Cumulative $^{14}CO_2$ evolution: 76.3% after 28 days	<u>Positive control</u> (aniline): 61.5% degradation after 28 days <u>Half-lives of 2-BNE</u>: DT_{50} = 0.00469 h (biotic assay) and 19 h (abiotic assay) Major metabolites (>10% TAR): 2-nitroethanol and nitromethane; Minor metabolites: Bromonitro-methane (≤4% TAR in biotic and ≤6% in abiotic assay)	[redacted] *et al.* 2022 (A.7.1.2.1.1-02)
[1] Test according to OECD criteria											

In the OECD TG 314B study by [redacted] (2012), rapid degradation of ^{14}C-Bronopol and mineralization to $^{14}CO_2$ was observed in the biotic mixtures. Within 1 minute, ^{14}C-Bronopol decreased to 63.9% of applied radioactivity (AR), and after 1 hour, only 1.2% ^{14}C-Bronopol remained. In the abiotic control mixture, ^{14}C-Bronopol was present at 100.3% of AR at the 1-minute sampling interval and declined to 1.3% of AR by the end of the test (Day 28). The similar degradation behaviour in both mixtures - the biotic and the abiotic ones – as well as the speed of degradation within the first minutes indicates that the initial removal of Bronopol was mainly driven by abiotic degradation processes. With regard to the test conditions in the study (pH 7.3±0.5, incubation temperature: 20±3 °C), hydrolysis may be the most relevant process here.

Emerging degradation products were identified by retention time match with analytical standards in HPLC assay with radiochemical detection. In the biotic mixtures, **2-hydroxymethyl-2-nitro-1,3-propanediol** (TNM) was identified as major transient metabolite. Its formation reached 23.2% of AR within 1 minute, and a maximum of 92.5% after 2 hours, before it declined to <1% of AR by Day 5 (retention time of 3.4 minutes). Besides TNM, only an unknown polar metabolite was detected but could not be identified. It reached 18.2% of AR on Day 3 and decreased to 2.8% of AR by Day 6 and was not detectable from day 6 on until test termination at day 28. Initially formed TNM was subsequently degraded to this unknown degradation product with a RT of 3.9 minutes. At the time of evolvement of the peak RT 3.9 minutes bronopol was almost completely degraded and the TNM concentration was at its maximum, hence TNM is very likely the major source of such peak at RT 3.9 minutes. The formation of 2-nitro-1,3-propanediol degradation product from TNM is the most likely degradation pathway. A half-life of 1.35 d at 20.55 °C was derived for this metabolite. There are three other peaks detected which correspond to three minor degradation products (< 10% initial activity). One of those, the metabolite at RT 6.1 minutes, was immediately analytically detected at test initiation at the first sampling point (1 minute) with 4.3% and was identified for the last time at the 30 minutes sampling interval with 5.8%. This metabolite was not detected anymore until test termination at day 28. Consequently, it was only analytically measurable for 30 minutes. Since it was immediately detectable, bronopol as the source for the metabolite at RT 6.1 minutes is very plausible. Given the almost immediate appearance and disappearance of that peak at 6.1 minutes, the formation of a highly biodegradable molecule is likely, such as 2-bromo-glycerol.

Regarding the metabolite at RT 3.9 minutes, it appears late in the test, that means that it is unlikely to be formed in the STP with the HRT considered there.

These proposed identified metabolites were evaluated for their potential ecotoxicological risks (OECD QSAR 4.5 and ECOSAR 2.2 if needed to complete the dataset). The ECHA PBT profiler, which is part of the toolbox, showed no alert according to PBT properties of the metabolites as the B criteria is not meth by both. Regarding 2-nitro-propane-1,3-diol (RT 3.9 min), the most sensitive species is estimated to be daphnia witch a chronic value of 6.09 mg/L and an acute value of EC50 5.14 mg/L. This toxicity is lower than Bronopol's. Related to the metabolite at RT of 6.1 min, which was only detectable for 30 min, the most sensitive species was estimated to be algae with a ChV 0.065 mg/L and an EC50 400 mg/L, also covered by parent toxicity. Further, and taking the short time of detection of 30 minutes into account, it can be stated that this metabolite will only be present in the STP and will not likely reach the environment. Hence no further assessment for these metabolites is needed.

In the abiotic mixtures, **2-bromo-2-nitroethanol** (2-BNE) was identified as major transient metabolite. It reached 14.5% of AR after 30 minutes, and a maximum of 55.3% of AR on Day 2, before it declined to 20.1% by Day 28. Besides 2-BNE several non-retained polar ^{14}C-compounds were detected and expected to be degradation products from hydrolysis, possibly formaldehyde, methanol and formic acid. They reached 3.5% of AR after 3 hours and 70.8% of AR as a maximum on Day 14. Recovery of total applied radioactivity was 84.7% at the end of the study. Non-linear regression analyses were performed for loss of parent compound and CO_2 production in the biotic mixtures. The average time for loss of 50% of ^{14}C-Bronopol (DT_{50} value) was approximately 2.99 h^{-1} (at

15 ºC) derived from k (20 ºC) of 5.01 h^{-1} from the study, while 50% CO_2 production occurred at approximately 65 hours at 15 ºC (k (15 ºC) = 0.0107 h^{-1}). As only one sludge was used in the OECD TG 314B study, as stated in section B.4.2, method 1 is the appropriate to derive the distribution in the STP.

Treatment	Process	Function	F-value	R^2	A (%)	K_1 (hrs^{-1})
Biotic	Loss of Parent	1st Order	111020	0.999	69.6 ± 0.22	5.01 ± 0.04

Biotic: average of biotic-1 and 2 replicates
F-value: fit value,
R^2: correlation coefficient,
A: initial parent concentration (%),
K_1: first order rate constant (hrs^{-1})

Treatment	Process	Function	F-value	R^2	A (%)	K_1 (hrs^{-1})
Biotic	CO_2 Production	1st Order	1421	0.989	74.0 ± 1.82	0.017 ± 0.001

Based on the findings of [redacted] (2012) it can be concluded that for scenarios where Bronopol is directly introduced into a Sewage Treatment Plant (STP) where biodegradation via microorganisms is the predominant pathway, the metabolite 2-BNE is not a relevant degradation product to consider. However, in cases where significant hydrolysis can occur prior to the entry into a STP, 2-BNE is a relevant transformation transient product.

Once in the STP, 2-BNE degrades very rapidly, according to the study from [redacted] (2022). In a biodegradation in activated sludge test, a very rapid transformation of 2-Bromo-2-nitro-ethanol (2-BNE) and mineralization to CO_2 was observed. Within 1 minute, 2-BNE declined to an average of 7.5% of total applied radioactivity (TAR), and after 15 minutes, it had disappeared from the biotic test solutions. Around 76% total CO_2 was produced by day 28 in the biotic assays. The average mass balance recovery ranged between 95% to 118%. The chemical analyses in radio-HPLC indicated that 2-BNE was transformed to NE (2-nitroethanol) as a first step, and NE was further degraded to NM (nitromethane) upon producing formaldehyde. NM is mineralized under formation of CO_2. Kinetic evaluation for degradation half-life determination was performed using nonlinear regression. The derived DT50s were 0.00469 h for 2-BNE, 0.275 h for NE and 35.8 h for NM. The DT50 for 2-BNE decay in the abiotic system is 19 h.

Further, considering the data from [redacted] *et al.*, degradation of 2-BNE in natural waters would be in the range of 8-19 h half-life (Kdeg 0.052-0.122 h-1), which supports the findings in the STP simulation test 314B with 2-BNE:

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.

According to WG-IV-2022:

- 2-BNE must be considered as transient product and does not need to be assessed as long as the emission takes place through STP. In such case 2-BNE is considered covered by Bronopol.
- Degradation product TNM should be assessed considering the worst case of all the parent degraded in the STP is transformed into this metabolite. This can be refined by considering the 314B test, and the CAKE simulation from such information. Hence, for risk assessment, the total fraction of parent converted to TNM is 99.75% and a

DT50 of 1.17 days at 20.55 ºC has been derived by CAKE simulation for this metabolite. A degradation rate in the STP for TNM = 0.01474/h at 15 ºC has been derived. A fraction of 14.46% of TNM will be degraded in the STP and hence, for risk assessment, the 85.54% of TNM formed will be released to surface water from the STP.

Value used in Risk Assessment	
Value/conclusion	Rapid degradation of Bronopol in wastewater, TNM and 2-BNE identified as main (transient) metabolites in the biotic and the abiotic test mixtures, respectively. K = 2.99 h^{-1} at 15 ºC. K (TNM) = 0.01474 h^{-1} at 15 ºC. K (2-BNE) = 68.43 h^{-1} at 15 ºC.
Justification for the value/ conclusion	The conclusion is based on the results of a valid GLP study according to OECD TG 314B, which is a suitable test method to investigate the aerobic degradation processes in the wastewater of a STP.

Anaerobic biodegradation

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biological sewage treatment, anaerobic biodegradation (Annex II, Title 1, Point 10.1.3.1b)
Justification	According to the use patterns of Bronopol (PT 2, 11 and 12), exposure to anaerobic conditions is unlikely. No anaerobic biodegradation study needs to be conducted.

STP simulation test

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biological sewage treatment, STP simulation test (Annex II, Title 1, Point 10.1.3.1c)
Justification	Endpoint was addressed by an OECD TG 314B study on Bronopol (see above "Aerobic degradation", Tab. A-110)

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.

The recommended test methods for this endpoint according to Guidance on the BPR: Volume IV, Part A (Version 1.3) are OECD TG 309, ISO Method 14592 or US-EPA OPPTS 835.3100 with non-adapted inoculum. The ready biodegradation submitted with [^{14}C]Bronopol (according to OECD TG 301B) essentially uses the same test system as the OPPTS 835.3100 (shake flask equipped with CO_2 trap). The only significant difference is that the OPPTS 835.3100 allows a higher inoculum concentration (in OECD TG 301B studies concentration of biosolids is limited to 30 mg/L).

Based on this, it would be expected primary degradation of the parent Bronopol molecule to be still very fast due to both hydrolytic and biodegradation processes, and the degradation products formed to be the same.

Further, considering the data from [redacted] *et al.*, during the hydrolysis test at 20 ºC, the DT50 obtained for Bronopol is around 0.11 d. From the photolysis experiment at 34 ºC the hydrolysis results (dark) is around 0.04 h (see table below, k deg 19.51-22.39 h^{-1}), considerably higher degradation rate, so it might be happening that some other degradation

is taking place and not only hydrolysis, in such test. The value of total degradation in natural waters at 12 ºC would be around 0.24 h. Actually, this is just an approximation, the tests were mainly exploring the abiotic degradation, hence the biotic degradation has not been explicity measured but it seems to be taking place to some extent. Hence this value is just supporting and should not be used for risk assessment.

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.

Hence, 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.

Table 82: 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
Supportive study			Bronopol		0.24 h (12 ºC)		[redacted] *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

Value used in Risk Assessment	
Value/conclusion	0.24 h (12 ºC)
Justification for the value/ conclusion	There is no experimentally derived value from an available study. Nevertheless, the publication available clearly shows that Bronopol will degrade not only abiotically, but biotically at a very high rate, in freshwater. This value is just supportive and not to be used for risk assessment.

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. 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. The recommended test methods for this endpoint according to Guidance on the BPR: Volume IV, Part A (Version 1.3) are OECD TG 309, ISO Method 14592 or US-EPA OPPTS 835.3100 with non-adapted inoculum. The ready biodegradation that we ran with [^{14}C]Bronopol (according to OECD TG 301B) essentially uses the same test system as the OPPTS 835.3100 (shake flask equipped with CO_2 trap). The only significant difference is that the OPPTS 835.3100 allows a higher inoculum concentration (in OECD TG 301B studies concentration of biosolids is limited to 30 mg/L). Based on this, we would expect primary degradation of the parent Bronopol molecule to be still very fast due to both hydrolytic and biodegradation processes, and the degradation products formed to be the same. 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	There are no direct releases of Bronopol into water. Only minor residual amounts of Bronopol will reach surface water indirectly via the STP due to the inherent biodegradability of Bronopol. 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

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). 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) 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 and [redacted] 1996

Seawater/sediment degradation study

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	Direct emission can be excluded as Bronopol is not used in or released into marine environments. Moreover, if Bronopol gets into a water-sediment-system, it does not adsorb to sediment to any relevant extent, but remains in the water phase where it is degraded via abiotic and biotic processes. Accordingly, there is no need in conducting a water/sediment degradation study in seawater.

A.4.1.1.3.4 Higher tier degradation studies in water or sediment

A.4.1.1.3.5 Biodegradation during manure storage

Data waiving	
Information requirement	Rate and route of degradation including identification of metabolites and degradation products, biodegradation during manure storage (Annex II, Title 1, Point 10.1.3.4)
Justification	According to the Guidance on the BPR: Volume IV, Part A (Version 1.3), a study on biodegradation in manure is needed for substances which are applied in animal housings and go to manure storage before release to the environment. As this is not the case for Bronopol, *i.e.*, it is not an intended use for Bronopol, a biodegradation study in manure is not necessary.

A.4.1.1.3.6 Biotic degradation in soil

A.4.1.1.3.6.1 Laboratory soil degradation studies

Aerobic 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). Hence, DT50 = 300 d (worst-case default value (table 6, ERA, 2017).
Justification	Bronopol is not directly released to soil when applied as recommended for the use patterns PT 2, 11 and 12. [redacted] (1992) showed that bronopol does not adsorb effectively to soil and rather is instable in this compartment. On the other hand, bronopol is highly water-soluble and mainly is distributed in the compartment water (> 99%), as shown by Mackay Level I calculation ([redacted] 2006): Media: air - biota - sediment(s) - soil - water Method: Calculation according Mackay, Level I Result: Distribution of bronopol in the different media: Air: 0.000238% Water: 99.9733% Soil: 0.0131% Sediment: 0.0133%; 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 and no soil degradation study needs to be conducted. The default value selected is based on: - Bronopol is a borderline case for readily biodegradability. - According to the supporting information regarding readily biodegradability (A7.1.1.2.1_01) bronopol was biotransformed by day 29 to 92 %. According to ECHA Guidance R7b: "when results of ready biodegradablity tests indicate that the pass level criterion is almost fulfilled, such results can be used as evidennce for inherent biodegradability. - In the inherent test the parent substance disappears completely at day 3 (supporting information). - The half-life of Bronopol due to degradation in natural waters (literature supporting information) is 0.03 h. If a DT50 for surface water is available, it may serve as basis for a DT50 in soil derived from table 6 as it is done with screening tests. - It has a very low Kp of 2.72 l/kg.

	Further, there is an on-going OECD TG 307 study which has been required to the applicants. The pre-test OECD TG 307 results gives a worst case DT50 of around 5 days in soil at 20 ºC (9.3 days at 12 ºC). This value cannot be used for further risk assessment at product level. This is being used only as supporting data at active substance level. References: [redacted] 1992 and [redacted] 2006 (IUCLID)

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 (PT 2, 11 and 12), 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 PT 2, 11 and 12. In addition, Bronopol is highly water-soluble and mainly distributed in the compartment water ([redacted] 2006) where it is susceptible to both hydrolysis and biodegradation. Consequently, no field studies on dissipation in soil are needed. Reference: [redacted] 2006, IUCLID

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] and [redacted]) 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 ([redacted], [redacted], [redacted]), 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 (2-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 2-Hydroxymethyl-2-nitro-1,3-propanediol (Tris(hydroxymethyl)nitromethane or TNM), Glycolic acid, Formic acid, Methanol (all <5%, 24 h) and 2,2-Dinitroethanol (<1%).

The decomposition of 2-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]). 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 TG 301:

- Modified OECD TG 301B test ([redacted]): 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]): 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]). 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 products are toxic to the environment and fulfil the criteria for classification as hazardous to the aquatic environment (2-BNE and TNM). 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 83: 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 TG 121 (HPLC method), GLP, Rel. 2	No soil	n.a.	log Koc = 1.0; Koc = 10	n.a.	n.a.	n.a.	n.a.		[redacted] 2002c (A7_1_3-01)
OECD TG 121 (HPLC method), GLP, Rel. 3	No soil	n.a.	log Koc = 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.** Comp. B 54.26 Comp. A (2-BNE) 24.85 Comp. C (TNM) 9.88 Comp. D 9.42	[redacted] 1992 (A7.1.3_01)
	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 (2-BNE); Component C was chromatographically similar to TNM

Adsorption of ^{14}C-Bronopol onto and desorption from four different soil types was investigated ([redacted] 1992), according to US EPA Pesticide Assessment 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 L/kg (geometric mean) was calculated for Bronopol. According to CLP, "a substance shall be considered to fulfil the mobility criterion (M) when the log Koc is less than 3", which is the case for Bronopol with a log Koc = 2.13, hence Bronopol is considered as moderately mobile.

Moreover, in the study of [redacted] (1992) 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 TNM) formed in the more acidic soils (loamy sand and clay loam).

In the two supporting studies ([redacted] 2002c, [redacted] 2000), 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).

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 (2-BNE, TNM, formaldehyde).

For 2-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 2-BNE, TNM and formaldehyde on soils and sediments is not expected.

Value used in Risk Assessment	
Value/conclusion	136 L/kg (Koc)
Justification for the value/ conclusion	The value was calculated based on the results of a valid GLP study with four soil types and identification of degradation products, as a geomean for all soil samples.

A.4.1.2.2 Higher tier soil adsorption studies

A.4.1.2.3 Volatilisation

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

A.4.1.3 Bioaccumulation

Measured aquatic bioconcentration

No data available.

Estimated aquatic bioconcentration

Data waiving	
Information requirement	Bioconcentration, estimation methods (Annex II, Title 1, Point 9.1.4.1) and experimental determination (Annex II, Title 1, Point 9.1.4.2)
Justification	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.

Measured terrestrial bioconcentration

Data waiving	
Information requirement	Bioconcentration, terrestrial (Annex II, Title 1, Point 9.6)
Justification	Bronopol is not intended for direct release to the terrestrial compartment. Therefore, exposure of terrestrial biota is considered negligible. In case of (unintended or indirect) release to soil, Bronopol is expected to remain in the pore water based on the high water solubility and the low log Pow. Measuring terrestrial bioconcentration is therefore not relevant.

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, 2-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 [redacted] (2006)[7]. 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	

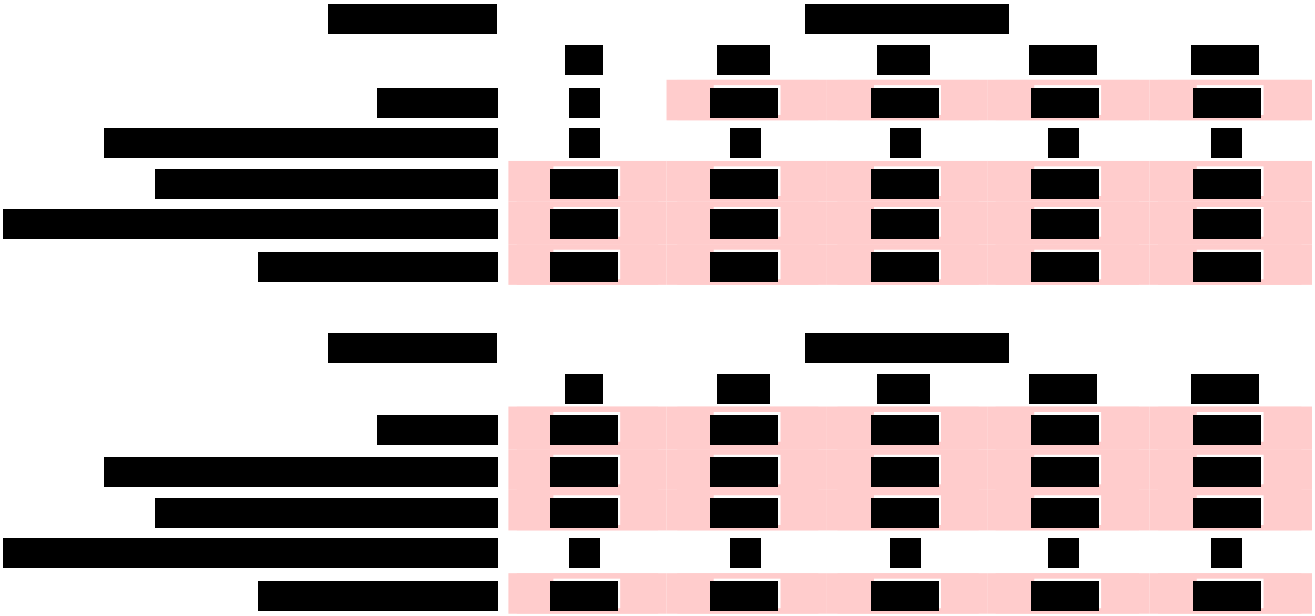
Due to the widespread use of Bronopol, it is to be expected that Bronopol was also used in the regions in Sweden where sampling was carried out. Therefore, if Bronopol would be persistent in the environment, findings above the LOD would have to be expected. This was the case for other biocidal active 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 (0.00048 mg/L).

There is another study available ([redacted] port) monitoring Bronopol in urine and paper pulp which demonstrate the degradation of the a.s. in each matrix. A start concentration of 50 ppm Bronopol was spiked to the synthetic urine in two different matrix; after 1 h, 15 ppm Bronopol were determined. After 24 h, the concentration of Bronopol was below the LOQ, < 5 ppm. This could be applicable to refine PT2 scenario for chemical toilets. Matrix 1 is synthetic urine according to Patent [redacted] which may contain a variety of organic compounds that in some way may influence decay pathway of Bronopol. In Matrix 2, synthetic urine according to DIN/EN 1616, there is just one defined organic compound present which is creatinine, as well as sodium sulphite present, rather reactive could have a decisive influence on breakdown pathway, but the complete mechanistic explanation of the different behaviour (major transformation product 2-BNE in matrix 1 and TNM in matrix

[7] Results from the Swedish Screening 2005. Subreport 2. Biocides (Swedish Environmental Research Institute)

2) is not provided, as this is just monitoring data for supporting information. The determination of the individual substances was done with HPLC method 1 for Bronopol, TNM, nitromethane, nitroethanol and with HPLC method 2 for Bromo-nitromethane (BNM) and 2-BNE. Accordingly, the calibrations were done with the corresponding HPLC method for each individual compound.



Regarding PT12 slimicides for paper industry, a start concentration of 50 ppm Bronopol was spiked to the paper pulp. After 1 h, 49 ppm Bronopol were determined. After 24 h, the concentration of Bronopol decreased to 27 ppm, so this supports the fact of the degradation of the a.s. in this scenario.

There is another report "[redacted] *et al.*, 2007"[8], where several analysis were performed in different sites in Nordic countries, including all environmental compartments, even sludge from industrial STPs in paper factories. Despite a high consumption of bronopol in the Nordic countries as well as within the EU, it was not found in any of the samples analyzed (below LOD of 0.1 µ/L in water and below LOD of around 20 ng/g dw in sludge). Bronopol undergoes rapid hydrolysis as well as biodegradation, which may explain its absence in the environmental matrix samples.

Other monitoring data come from NORMAN database:

Sampling date	Compartment	LOQ	LOD	Country	Sampling sites	Organisation	Result
2012	Surface water	0.13 µg/l		Switzerland	5		< LOQ
2018	Surface water	0.0038 µg/l	0.0013 µg/l	Montenegro	5		< LOD
2018	Surface water	0.0038 µg/l	0.0013 µg/l	Ukraine	3		< LOD
2018	Sediments	15.2 µg/kg dw	5 µg/kg dw	Montenegro	3		< LOD

[8] [redacted], [redacted], [redacted], [redacted], [redacted], [redacted] & [redacted] 2007. Bronopol, Resorcinol, m-Cresol and Triclosan in the Nordic Environment. Nordic Council of Ministers, Denmark. TemaNord 2007:585. 81 s.

2018	Groundwater	0.0038 µg/l	0.0013 µg/l	Ukraine	4		< LOD
2013	Groundwater	0.0077 µg/l		Switzerland	9	Swiss Federal Institute of Aquatic Science and Technology (EAWAG), CH	< LOD
2017	Municipal wastewater	0.003 µg/l	0.001 µg/l	Romania, Serbia, Croatia, Slovenia, Hungary, Slovakia, Germany, Czech Republic, Austria	12		< LOD

A.4.2. Effects on environmental organisms

A.4.2.1 Atmosphere

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.4.2.2 Toxicity to sewage treatment plant (STP) microorganisms

Inhibition of microbial activity (aquatic)

Table 84: 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. 3	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 TG 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 TG 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). 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). The test was performed with a guidance equivalent to OECD TG 209. The test is considered as supporting information due to certain deficiencies. First of all, there is no preliminary study, but there are more deficiencies, which make the study less reliable than the one from Forster, such as no details are given in the report on the composition of the culture medium except 'synthetic medium' and no confidence limits reported for the given EC50.

Both studies are based on nominal concentrations, which in case of Bronopol means that the real concentrations might be quite different than nominals. This would lead to the application of paragraph 56 in OCDE 209, which states it is enough with an order of magnitude for an EC50, in this case 10-100 mg/L. Hence the most reliable test results are preferred to be used for risk assessment.

In a further OECD TG 209 study ([redacted] 2002), 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). 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.

Value used in Risk Assessment	
Value/conclusion	43 mg/L (2.5h-EC_{50})
Justification for the value/ conclusion	The value was determined in a valid GLP study according to OECD TG 209 on a test substance with a purity >99% and can therefore be considered as reliable.

A.4.2.3 Aquatic compartment

A.4.2.3.1 Freshwater compartment

Acute/short-term toxicity (freshwater)

Table 85: 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 TG 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)

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
OECD TG 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 TG 203, non-GLP, Rel. 2	*Oncorhynchus mykiss*	Mortality	2-Hydroxymethyl-2-nitro-1,3-propanediol (trade name: Tris-hydroxymethyl-nitromethane) (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 TG 203 (1992), GLP, Rel. 2	*Oncorhynchus mykiss*	Mortality	2-bromo-2-nitroethanol (2-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)
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 TG 202, GLP, Rel. 3	*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,	*Daphnia magna*	Immobility	2-	static	48 hours	EC_{50} = 80 mg/L	Nominal conc.	[redacted] 1989

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
Rel. 2			Hydroxymethyl-2-nitro-1,3-propanediol (trade name: Tris-hydroxymethyl-nitromethane) (TNM)					(A7_4_1_2-03)
OECD TG 202 (2004), GLP, Rel. 2	*Daphnia magna*	Immobility	2-bromo-2-nitroethanol (2-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 TG 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 TG 201 (1984), GLP, Rel. 3	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	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)
OECD TG 201	*Desmodesmus*	growth	Bronopol	static	96 hours	72h-E_rC_{50} = 0.5	measured conc.	[redacted] 1998

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
(1984), US EPA OPPTS 850.5400, GLP, Rel. 2	*subspicatus* (formerly *Scenedesmus subspicatus*)	inhibition, Algistatic effects			plus 7 days recovery period	mg/L (geomean)		(A7.4.1.3_02)
OECD TG 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} = 0.109 mg/L	geometric mean measured concentration	[redacted] 2006b (A7_4_1_3-02)
OECD TG 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 TG 201 (1984), GLP, Rel. 3	*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 TG 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (*Pseudokirchneriella subcapitata,* formerly *Selenastrum capricornutum*)	Growth inhibition	2-bromo-2-nitroethanol (2-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 TG 201	*Raphidocelis*	Growth	2-	static	96 hours	72h-E_rC_{50} > 4.5	Mean measured	[redacted] 2002

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
(1984), Directive 92/69/EEC Method C.3, GLP, Rel. 2	*subcapitata* (*Pseudokirchneriella subcapitata,* formerly *Selenastrum capricornutum*)	inhibition	Hydroxymethyl-2-nitro-1,3-propanediol (trade name: Tris-hydroxymethyl-nitromethane) (TNM)			mg/L	conc.	(A7_4_1_3-06)
OECD TG 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 TG 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 TG 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. 3	*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 TG 221 (proposal April 2004), US EPA OPPTS 850.4400,	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)

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LC/EC_{50}		
GLP, Rel. 2								

Description of the available acute toxicity studies

As a general remark, the reliability index for the studies with Bronopol, which disappears very rapidly and hence the nominals are not reliable, will be 3 for such short-term studies with no measured concentrations. Regarding degradation product TNM, the half-life of this metabolite is not so low. Hence, the nominals can be accepted to derive a PNEC for TNM. Actually, the ENV WG agreed to the $PNEC_{water}$ value for TNM, which is based on the algae endpoint (measured concentrations) and an AF =1000 as described in Table 18 in BPR guidance 2017 vol IV, B+C: "At least one short-term L(E)C50 from each of three trophic levels (fish, aquatic invertebrate and algae)", by considering valid the studies with fish and daphnia.

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 (Hill 1984) and 4.28, 7.13, 11.9, 19.8, 33.0 and 55.0 mg/L (2005) according to US EPA Guideline OPP 72-1 and OECD TG 203, respectively. While in the study of (1984) the LC50 was 41.2 mg/L and the NOEC 23 mg/L (both based on measured concentrations), in the study of (2005) 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) and 5.5, 10, 18, 33 and 60 mg/L (2006a) according to US EPA Guideline OPP 72-1 and OECD TG 203, respectively. In the study by (1984), 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), 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). 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 TNM (1973), with no GLPs or analytical confirmation of the concentration tested due to the year of performance. The test is valid with restrictions, but clearly showing a low effect of TNM 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 TNM to fish.

A study on acute toxicity of 2-BNE to *Oncorhynchus mykiss* following the OECD TG 203 guideline was performed (2012). 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 hours 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) between two consecutive treatments and the calculation method used, *i.e.*, EC_{50} as geometric mean of EC_0 and EC_{100} ([redacted] 2000).

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) 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 EC 50 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 Williams (1981) was conducted by using methods that are comparable to OECD TG 202 and the [redacted] 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 test is not considerer reliable.

The study of [redacted] (1998) 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. 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). 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) as guideline study with well characterised test material was preferred to the one by [redacted] (1981).

Regarding metabolites, a study on acute toxicity to *Daphnia magna* following the OECD TG 202 guideline was performed with 2-BNE ([redacted] 2012). 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 TNM ([redacted], 1989) 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. Some of the studies did not include analytical measurements. Taking into account the rapid hydrolysis, the studies with no analytical measurements are considered as supporting information.

The key study with *Desmodesmus subspicatus* ([redacted] 2006a) 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 GD 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). 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 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, hence the NOEC is not reliable, and the test is considered as supporting information. The test with *Subcapitata* (test a) is considered as supporting information only, due to the lack of analytical measurements.

[redacted] 2002 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 TG 201 study with *Desmodesmus subspicatus* was conducted ([redacted] 1998), 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 and 2006c). 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) 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 Desjardins (2006b) 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). 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, [redacted] 1998). The growth inhibition test of [redacted] (1998) 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), for comparability. Nevertheless, the study from [redacted] is considered as supporting information as no analytical measurements are provided. In the test from [redacted], 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 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*)	TNM	410 mg/L (96h-LC_{50})
	2-BNE	3 mg/L (96h-LC_{50})
Water flea (*Daphnia magna)*	TNM	80 mg/L (48h-EC_{50})
	2-BNE	0.38 mg/L (48h-EC_{50})
Green algae (*Raphidocelis subcapitata)*	TNM	>4.5 mg/L (72h-EC_{50})
	2-BNE	0.109 mg/L (72h-EC_{50}) based on TWA

The test with TNM ([redacted] 2002) 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 2-BNE ([redacted] 2012), 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 2-BNE. 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 2-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, 2-BNE is considered to degrade very rapidly in the STP, according to an OECD TG 314 simulation test.

For aquatic plant *Lemna gibba* a test was conducted ([redacted], 2006e). 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).

Value used in Risk Assessment	
Value/conclusion	Lowest acute endpoint 0.0073 mg/L (72h-E_rC_{50}) as geometric mean measured concentrations. Chronic data available, therefore not relevant.
Justification for the value/ conclusion	The green algae *D. subspicatus* has shown to be the most sensitive species among the aquatic test organisms. Short-term toxicity is assessed based on the EC_{50} values. The value to be used in Risk Assessment was derived in a valid GLP guideline study and calculated based on geometric mean measured concentrations.

Chronic/long-term toxicity (freshwater)

Table 86: 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 TG 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 TG 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 TG 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 TG 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]								

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)		
OECD TG 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-NOEC=0.0026 mg/L72h-EC10=0.0048 mg/L, calculated and used for PNEC derivation)	geometric mean measured concentration	[redacted] 2006a (A7_4_1_3-01)
OECD TG 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 TG 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 TG 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 TG 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 TG 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata*	growth inhibition	Bronopol	static	96 hours	72h-NOEC=0.10 mg/L	Nominal conc., no analytical	[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
				Design	Duration	LOEC/NOEC/EC_{10} (specify the value)		
	(formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)						monitoring	
OECD TG 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 TG 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 TG 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. 1 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 TG 201 (1984), GLP, Rel. 2	*Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	Growth inhibition	2-bromo-2-nitroethanol (2-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 TG 201	*Raphidocelis*	Growth	TNM	static	96 hours	E_rC_{10} = 0.572 mg/L	Mean	[redacted] 2002

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)		
(1984), Directive 92/69/EEC Method C.3, GLP, Rel. 2	*subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	inhibition					measured conc.	(A7_4_1_3-06)
Other aquatic plants								
OECD TG 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-days juvenile growth test according to OECD TG 215 ([redacted] 2007) and a 49-days flow-through test according to OECD TG 210 ([redacted] 1996). The observed test parameters for the concentration ranges of nominal 0.32 to 32 mg/L ([redacted] 2007) and 2.25 to 40 mg/L ([redacted] 1996) were mortality, sublethal effects, and body weight.

In the study of [redacted] (2007) 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 TG 210 ([redacted], 1996). 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 (2-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) and nominal 0.017 to 1.7 mg/L ([redacted] 1992) 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), reproductive efficiency and growth of adult daphnids were the most sensitive parameters, both significantly reduced in the highest test concentration of 0.109 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 (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), 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 mg/L measured concentrations). Results should be expressed as mean 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 0.0026 mg a.s./L or EC_{10} of 0.0048 mg/L (based on mean measured concentration), determined for *D. subspicatus* in the OECD TG 201 study of [redacted] (2006a). 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.

Value used in Risk Assessment	
Value/conclusion	0.0048 mg/L (72h-EC_{10}, *D. subspicatus*)
Justification for the value/ conclusion	The green algae *D. subspicatus* has shown to be the most sensitive species among the aquatic test organisms. Long-term toxicity is assessed based on the NOEC/EC10 values. The value to be used in Risk Assessment was derived in a valid GLP guideline study and calculated based on mean measured concentrations of the active substance. The EC10 is chosen better than the NOEC because it is considered as a more accurate endpoint derived from a robust statistical analysis. It is based on geometric mean measured concentrations.

A.4.2.3.2 Sediment compartment (freshwater)

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, 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, 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, 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, 11 and 12), exposure to sea sediment is unlikely.

A.4.2.4 Terrestrial compartment

Toxicity to terrestrial organisms, acute/short-term tests

Table 87: Summary table - acute/short-term terrestrial toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Organic matter (mg/Kg dw)	Results			Remarks	Reference
				Design	Duration		NOEC	LC/EC 10	LC/EC50		
Earthworm/soil-dwelling non-target invertebrates											
OECD TG 207 (1984), GLP, Rel. 2 Key	*Eisenia foetida*	Mortality	Bronopol	Application in soil	14 days	Not stated	12.8 mg/kg dw	LOEC= 32 mg/kg dw	>500 mg/kg dw	Nominal concentrations	[redacted] 2006 (A7.5.1.2 _ 01)
OECD TG 207 (1984), GLP, Rel. 2	*Eisenia foetida*	Mortality, wet weight	Bronopol	Application in soil	14 days	Not stated	250 mg/kg dw	LOEC= 500 mg/kg dw	>1000 mg/kg dw	Nominal concentrations	[redacted] 2007 (A7_5_1_2)
Soil microflora											
OECD TG 217, GLP, Rel. 1	Aerobic soil microflora in silty	Carbon transformation, soil	Bronopol	Application in soil, incubatio	28 days	OC content: 1.39±0.2	Not stated	70 mg/kg dw	>1186 mg/kg dw	Initial microbial biomass:	[redacted] 2007a (A7.5.1.1 01)

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Organic matter (mg/Kg dw)	Results			Remarks	Reference
				Design	Duration		NOEC	LC/EC 10	LC/EC50		
	sand (acc. To German DIN)	respiration		n in the dark, sampling times: 0, 7 and 28 days		4% dw				285.8 mg/kg dry soil matter	
OECD TG 217 (2000), GLP, Rel. 1 Key	native microflora in soil sample used (sandy loam soil)	CO_2 respiration	Bronopol	Application in soil using quartz sand carrier, incubation in the dark, sampling times: 0 (less than 3 hours) and 28 days	28 days	OC content: 1.02±0.15% dw	Not stated	10.4 mg/kg dw	104.4 mg/kg dw	Initial microbial biomass: 1.0% (percent of TOC) 100.6 mg microbial C/kg dry soil	[redacted] 2007 (A7_5_1_1-01)
OECD TG 216, GLP Rel. 1	Aerobic soil microflora in silty sand (acc. To German DIN)	Nitrate production	Bronopol	Application in soil, incubation in the dark, sampling times: 0, 7 and 28 days	28 days	OC content: 1.39±0.24% dw	Not stated	25 mg/kg dw	679 mg/kg dw	Initial microbial biomass: 285.8 mg/kg dry soil matter	[redacted] 2007b (A7.5.1.1_02)
OECD TG 216 (2000), GLP, Rel. 1	native microflora in soil	Nitrification	Bronopol	Application in soil using	28 days	OC content 1.02±0.1	Not stated	11.5 mg/kg dw	78.1 mg/kg dw	Initial microbial biomass:	[redacted] 2007 (A7_5_1_1-01)

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Organic matter (mg/Kg dw)	Results			Remarks	Reference
				Design	Duration		NOEC	LC/EC $_{10}$	LC/EC$_{50}$		
Key	sample used (sandy loam soil)			quartz sand carrier, incubation in the dark, sampling times: 0 (less than 3 hours) and 28 days		5% dw				1.0% (percent of TOC) 100.6 mg microbial C/kg dry soil	
Non-target plants											
non-standardized seedling emerge test, non-GLP, Rel. 3 supportive	*Lycopersicum esculentum*	Germination	Bronopol	Water agar containing serial dilutions of Bronopol	7 days	Not stated				Toxic effects were observed at 1000 µg/mL, however, no further details on the degree of effects (EC_x) and the endpoint investigated were given.	[redacted] 1986 (A7.5.1.3 _01)
OECD TG 208 (2006), GLP, Rel. 1 Key	(1) *Brassica napus* (Oilseed rape), (2) *Lactuca sativa*	Emergence, dry weight	Bronopol	Application into soil, incubation in a greenhouse,	15 days	Not stated	Emergence: (1 and 2) 82 mg/kg dw; (3) 642 mg/kg dw	Not stated	Emergence: (1 and 3) >642 mg/kg dw; (2) 227 mg/kg dw Growth	The so measured concentrations were 50.9, 81.9, 152.9, 368.6, and	[redacted] 2007 (A7_5_1_3-01)

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Organic matter (mg/Kg dw)	Results			Remarks	Reference
				Design	Duration		NOEC	LC/EC $_{10}$	LC/EC$_{50}$		
	(Lettuce), (3) *Lolium perenne* (perennial ryegrass)			artificial light in the morning and evening to obtain 16:8 light/dark cycle, assessment on Day 7 and 15			Growth (dry weight): (1+2) 82 mg/kg dw; (3) 369 mg/kg dw		(dry weight): (1) 222 mg/kg dw; (2) 227 mg/kg dw; (3) >642 mg/kg dw	642 mg/kg test item.	
Bees and other (non-target) arthropods											

Acute (short-term) toxicity to earthworm

The acute toxicity of Bronopol to earthworms was studied with *Eisenia foetida* according to OECD TG 207 ([redacted] 2006, [redacted] 2007). Adult earthworms (with visible clitella) were exposed for 14 days to Bronopol-treated artificial soil. The test concentrations were 12.8, 32, 80, 200 and 500 mg/kg ([redacted] 2006) and 31.25, 62.5, 125, 250, 500, and 1000 mg/kg ([redacted] 2007) dry weight soil. The animals were checked for mortality after 7 and 14 days and for body weight at the start and at the end of the exposure.

In the study by [redacted] (2006) the lowest concentration of 12.8 mg/kg d.w. was defined as NOEC and 14d-LC_0, respectively, as the mortality of 8% at test end is below the maximum acceptable control mortality of 10% stated in the guideline. Control mortality was 5% confirming the validity of the study. The second lowest concentration of 32 mg/kg d.w. was consequently designated as LOEC and the LC_{50} and LC_{100} were both determined to be > 500 mg/kg d.w. since the effect level in the highest test concentration after 14 days was below 50% for mortality as well as for body weight change. Sublethal effects like reduced activity were observed in the two highest test concentrations of 200 and 500 mg/kg d.w.

No effects (lethal/sublethal) were observed in the second study by [redacted] (2007) up to concentrations of 250 mg/kg d.w. which was consequently defined as NOEC. In the next higher concentration of 500 mg/kg d.w. (LOEC) one earthworm died within the test period, but sublethal effects were observed (worms moving on the glass wall) and a body weight change of -15% at the end of the test. Since the effect level in the highest test concentration (1000 mg/kg d.w.) after 14 days was below 50% for mortality as well as for body weight change, the LC_{50} and LC_{100} were determined to be > 1000 mg/kg d.w. Validity of the study was confirmed by 0% mortality in the control. The results are based on nominal concentrations based on a range-finding test which is not reported.

Acute (short-term) toxicity to soil microorganisms

The adverse effects of Bronopol on aerobic soil microorganisms were investigated by means of two carbon transformation tests according to OECD TG 217 ([redacted] 2007a, [redacted] 2007), which measures the carbon transformation by the glucose induced soil respiration, and two nitrate production tests according to OECD TG 216 ([redacted] 2007b, [redacted] 2007), where lucerne meal was used as source of nitrogen. The soil was defined as silty sand according to German DIN ([redacted] 2007a, 2007b) and sandy loam ([redacted] 2007). The test mixtures were incubated up to 28 days in the dark at a temperature of 20 ± 2 °C. Sampling times were on Day 0 and 28, and for the studies of [redacted] (2007a, 2007b) additionally on Day 7. At each time point, 3 samples per test concentration were taken. All studies were considered valid as the deviation in the control samples was below the threshold from the respective guideline.

The following nominal concentrations in soil were tested in the OECD TG 217 studies: 10, 26, 68, 178, 457 and 1186 mg/kg (Schwarz 2007a) and 10, 33, 111, 333, and 1000 mg/kg ([redacted] 2007) dry matter soil. Glucose [redacted] respiration rates were measured for 12-18 consecutive hours. The calculated respiration rate was expressed as mg O_2 released/kg dry matter soil/h ([redacted] 2007a) and mg CO_2 evolved/kg dry soil equivalents/h (Völkel 2007), respectively. The resulting EC_{10} values on carbon transformation after 28 days [redacted] to Bronopol were 70 mg/kg ([redacted] 2007a) and 10.4 mg/kg ([redacted] 2007) dry matter soil; the corresponding EC_{50} value after 28 days was > 1186 mg/kg ([redacted] 2007a) and 104.4 mg/kg ([redacted] 2007) dry matter soil. The deviation of glucose-induced respiration in the blank controls was < 15% at the end of the exposure, confirming the validity of the test.

The following nominal concentrations in soil were tested in the OECD TG 216 studies: 10, 26, 68, 175, 458, 1188 mg/kg ([redacted] 2007b) and 10, 33, 111, 333, and 1000 mg/kg ([redacted] 2007) dry matter soil. In the study of [redacted] (2007b) the content of nitrate in aqueous soil extracts was determined by means of ion chromatography using an IC system Dionex DX 120 apparatus, while in the study of [redacted] (2007) the content of nitrite and nitrate in 2 M KCl extracts of soil samples were determined by means of a Flow Injection Analyser. The percentage of inhibition of the nitrate production in the treated soil samples versus untreated controls was calculated. The EC_{10} value on nitrogen transformation after

28 days exposure to Bronopol was 25 mg/kg ([redacted] 2007b) and 11.5 mg/kg ([redacted] 2007) dry matter soil; the corresponding EC_{50} value after 28 days was 679 mg/kg ([redacted] 2007b) and 78.1 mg/kg ([redacted] 2007) dry matter soil.

Acute (short-term) toxicity to non-target plants

The toxicity of Bronopol on Tomato plants (*Lycopersicum esculentum*) was investigated in a non-standardized seedling emerge test using a test duration phase of 7 days ([redacted] 1986). Tomato seeds were germinated on water agar containing serial dilutions of Bronopol ranging from 2 to 100000 µg/mL at 25 °C in the dark. After 7 days, percentage of germination, root and shoot length was determined. Toxic effects on Tomato plants were observed at 1,000 µg/mL, however, no further details on the degree of effects (EC_x) and the endpoint investigated were given. This test is considered as supporting information, as it is a scientific publication and no further checking of the test quality is possible.

Further, a seedling emergence test according to OECD TG 208 ([redacted] 2007) was conducted under GLP conditions to investigate the effects of 15 days exposure to Bronopol on the emergence and growth of oilseed rape (*Brassica napus*), lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*) seeds. Bronopol was incorporated into soil uniformly via stock solutions which were prepared in purified water not longer than 10 min before application to soil samples. Analytical confirmation of the test concentrations of nominal 37.5, 75, 150, 300, and 600 mg/kg dry soil was conducted using LC/MS methodology. The analytical recoveries ranged from 102% to 136% of nominal, therefore the nominal concentrations were adjusted by the percent recoveries and the measured concentrations used for determination of the endpoints were 50.9, 81.9, 152.9, 368.6, and 642 mg/kg test item on dry soil. The monocotyledonous perennial ryegrass was the least sensitive species with EC_{50} values above the highest test concentration of measured 642 mg/kg d.w., with no effect for all parameters (emergence, mortality rate and growth). From the two dicotyledonous species tested, the emergence of the oilseed rape seeds (EC_{50} >642 mg/kg d.w.) was less affected than the emergence of lettuce seeds (EC_{50}=227 mg/kg d.w.). With respect to mortality and growth, both species were equally affected and the EC_{50} values for these parameters were comparable (growth EC_{50} was 222 mg/kg for oilseed and 227 mg/kg for lettuce, and mortality EC_{50} 364 and 469 mg/kg, respectively).

Value used in Risk Assessment	
Value/conclusion	78.1mg/kg dw (EC50, soil microflora)
Justification for the value/ conclusion	Not relevant, as chronic data are available.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

Toxicity to terrestrial organisms, chronic/long-term tests

Table 88: Summary table chronic/long-term terrestrial toxicity

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint/ Type of test	Test material	Exposure		Results	Remarks	Reference
				Design	Duration	LOEC/NOEC/EC10 (specify the value)		
Earthworm/soil-dwelling non-target invertebrates reproduction								
OECD TG 222 (2004), GLP, Rel. 2	*Eisenia foetida*	Mortality, Body weight change, Reproduction	Bronopol	Application in soil, incubation with 16:8 light/dark cycle, first assessment after 4 weeks, second assessment after 8 weeks (test end)	56 days	Mortality: NOEC=500 mg/kg dw EC10=618.3 mg/kg dw Body weight change: NOEC=500 mg/kg dw EC10=377 mg/kg dw Reproduction: NOEC=62.5 mg/kg dw EC10=61.5 mg/kg dw; Nominal concentrations reported, no analytical verification	EC10 values esp. for reproduction are not reliable in a scientific context as the width between lower and upper confidence limit at 95% CI is too large => EC10 value to be carefully considered for any further assessments	[redacted] 2021 (A7.5.2.1_01)
Soil microflora								
OECD TG 217, GLP, Rel. 1	Aerobic soil microflora in silty sand (acc. to German DIN)	Carbon transformation, soil respiration	Bronopol	Application in soil, incubation in the dark, sampling times: 0, 7 and 28 days	28 days	70 mg/kg dw	Initial microbial biomass: 285.8 mg/kg dry soil matter	[redacted] 2007a (A7.5.1.1_01)

OECD TG 217 (2000), GLP, Rel. 1 Key	native microflora in soil sample used (sandy loam soil)	CO_2 respiration	Bronopol	Application in soil using quartz sand carrier, incubation in the dark, sampling times: 0 (less than 3 hours) and 28 days	28 days	10.4 mg/kg dw	Initial microbial biomass: 1.0% (percent of TOC) 100.6 mg microbial C/kg dry soil	[redacted] 2007 (A7_5_1_1-01)
OECD TG 216, GLP Rel. 1	Aerobic soil microflora in silty sand (acc. To German DIN)	Nitrate production	Bronopol	Application in soil, incubation in the dark, sampling times: 0, 7 and 28 days	28 days	25 mg/kg dw	Initial microbial biomass: 285.8 mg/kg dry soil matter	[redacted] 2007b (A7.5.1.1_02)
OECD TG 216 (2000), GLP, Rel. 1 Key	native microflora in soil sample used (sandy loam soil)	Nitrification	Bronopol	Application in soil using quartz sand carrier, incubation in the dark, sampling times: 0 (less than 3 hours) and 28 days	28 days	11.5 mg/kg dw	Initial microbial biomass: 1.0% (percent of TOC) 100.6 mg microbial C/kg dry soil	[redacted] 2007 (A7_5_1_1)
Non-target plants								
No guideline study, non-GLP Rel. 3	*Cattleya aurantiaca*	Development (growth index)	Bronopol	Germinated on Knudsons C medium and seedlings were then transferred to the test media under aseptic conditions in 100 mL bottles at 22±2 °C and a day-night-regime of 12:12	3 months	No data	At 500 ppm Bronopol, the seedlings died.	[redacted] 1979 (A7.5.1.3 _02)

				h at 0.8 mw/cm^2 light intensity				
Bees and other (non-target) arthropods								

The toxic effects of Bronopol on survival, growth and reproduction of the earthworm *Eisenia fetida* were assessed during a test period of 56 days in a GLP-study based on OECD TG 222 ([redacted] 2021). The test item was homogeneously mixed into artificial soil at the nominal test concentrations of 31.3, 62.5, 125, 250 500 and 1000 mg/kg dry soil. No analytical monitoring was performed, and thus the results were based on nominal concentrations. Compared to the control where the body weight increased by on average 64%, the increase in the test concentrations were lower following a dose-response-relationship (except for treatment 125 mg/kg d.w.) with a sharp decline in the highest test concentration where the body weight increased only by 12% over the test period. The same observations (concentration-dependent decline) were made for reproduction where only 2% of the control value were achieved in the highest test concentration. However, the variance between the replicates was high due to the high mortality of the adult worms in two of four replicates. The high variance impaired the statistical evaluation by leading to broader confidence limits and thus weakening the reliability of the calculated EC_{10} values. Therefore, these values should be considered carefully.

In a further long-term study, seeds of the orchid *Cattleya aurantiaca* were germinated on Knudsons C medium and seedlings were then transferred to the test media under aseptic conditions in 100 mL bottles at 22±2 °C and a day-night-regime of 12:12 hrs at 0.8 mw/cm^2 light intensity ([redacted] 1979). The development of the plants (growth index) was measured after 3 months according to the growth index of Spoerl (average of 5 replicates) after exposure to 0, 50, 250 and 500 ppm Bronopol. Growth indices were as follows: 557±27, 547±12 and 520±6 at 0, 50 and 250 ppm. At 500 ppm Bronopol, the seedlings died and therefore, no growth index could be determined. Since the study was neither performed under GLP conditions nor according to any guideline, it was classified as supporting information not to be used in risk assessment.

The tests according to OECD TGs 216 and 217 are considered as long-term assays with provided reliable NOEC/EC10, as described in the acute toxicity section.

Value used in Risk Assessment	
Value/conclusion	10.4 mg/kg dw (28d-EC10, soil microflora)
Justification for the value/ conclusion	Soil microorganisms are considered the most susceptible species and, as the respective studies last for 28 days, the EC_{10} value is the preferred endpoint. It was derived in a valid GLP-study according to OECD TG 217 and can therefore be considered reliable. Studies according to OECD TG 217 can cover both acute and chronic toxicity assessments, hence the EC_{10} determined in the OECD TG 217 study should be used in risk assessment.

A.4.2.5 Groundwater

No data available.

A.4.2.6 Birds and mammals

Table 89: Summary table – toxicity to birds and mammals

Method, Guideline, GLP status, Reliability, Key/supportive study	Species	Endpoint / Type of test	Test material	Exposure		Results [mg a.i./kg bw or feed]			Remarks	Reference
				Design	Duration	LD/LC 50	LOEL/ LOEC	NOEL/ NOEC		
OPPTS 850.2100, GLP, Rel. 2, supportive	Mallard duck, *Anas platyrhynchos*	mortality / acute	Bronopol	single oral application	14 days post treatment	>56	>56	56	dose-response; regurgitation immediately after dosing at >56 mg a.s./kg bw	[redacted] 2005 (A7_5_3_1_1-01)
US EPA 71-2 (1983), OPPTS 850.2200, GLP, Rel. 1, Key	Bobwhite quail, *Colinnus virginianus*	Mortality, body weight, feed consumption, pathological changes / subacute	Bronopol	Application via diet, dosing was achieved by dietary inclusion for 5 days.	5d + 3d pre-, 4 d post-treatment	5d-LC50: 4,488	Not given	Not given	Alternative 5d-LC50: 7,379 (if bullied birds are excluded)	[redacted] 1984a (A7.5.3.1.2_01_a), [redacted] 1984a (A7.5.3.1.2_01_b), [redacted] 1984b (A7.5.3.1.2_01_c)
US EPA 71-2 (1983), OPPTS 850.2200, GLP, Rel. 1, supportive	Mallard duck, *Anas platyrhynchos*	Mortality, body weight, feed consumption, pathological changes / subacute	Bronopol		5d + 3d pre-, 4 d post-treatment	>10,000	Not given	Not given		[redacted] 1984b (A7.5.3.1.2_02_a), [redacted] 1984c (A7.5.3.1.2_02_b)

In an acute oral toxicity test with mallard duck ([redacted] 2005), mortalities were not found at any dosage between 14 and 113 mg a.s./kg bw. However, several birds of the highest treatment group regurgitated within 15 minutes after dosing preventing higher dosing. With regard to this effect, LC_{50} was determined as greater than the highest test dose without significant regurgitation. It could not be exactly determined due to regurgitation of the test substance, which however could not be influenced by the test design.

The subacute toxicity of Bronopol to Bobwhite quail (*Colinnus virginianus*) and Mallard duck (*Anas platyrhynchos*) was investigated according to US EPA guideline 71-2, OPPTS 850.2200 ([redacted] 1984, [redacted] 1984). After 3 days pre-treatment, young birds were exposed for 5 days to nominal dietary concentrations of 100, 500, 1,000, 5,000 and 10,000 ppm, followed by a 4-day observation period on untreated feed. Analytical measurements of Bronopol in the chick diet yielded recoveries between 89 and 118% for Bobwhite quail and between 90 and 133% for Mallard duck. Ten birds were allocated to each dose level, including 3 control groups. Observations for mortality and bird health were made daily; group mean body weights were recorded on day -3, 0, 5, 8 and 9. Group mean food consumption was examined for day -3 to -1, 1 to 5 (daily) and 6 to 9. All birds were inspected post-mortem at death or termination for gross pathological changes.

For Bobwhite quail, in one of the control groups, 10% mortality (1 bird/10) was observed, however, this bird showed signs of bullying. No mortality was seen in the other two control groups. In the treatments 100 and 500 ppm, mortality was also 0%, whereas at 1,000 ppm, 20% mortality was recorded (10% if the bird which showed signs of bullying was excluded). At 5,000 ppm, mortality was 30% (0% if bullied birds were excluded, all dead birds showed signs of bullying), and at 10,000 ppm, mortality was 90% (60% for non-bullied birds). Birds in treatment 10,000 ppm were subdued on day 5. Body weights were within normal limits, no treatment-related differences in food consumption were apparent. Some birds that died during treatment and post-treatment periods, showed evidence of bullying.

For mallard duck, a single case of mortality was reported for the 10,000 ppm group on day 5. In the 100, 500 and 10,000 ppm groups, birds were subdued up to day 6. In the 5,000 and 10,000 ppm groups, birds showed unsteadiness, huddling and subdued behaviour over day 2 to 6. A treatment-related reduction in mean body weight increase over day 0 to 5 was observed in the 5,000 and 10,000 ppm groups. For the remaining groups, body weight changes were within normal limits. A dose-related decrease in food consumption was observed at 5,000 and 10,000 ppm Bronopol over days 1 to 5. Further variabilities in food consumption were within normal limits. In the 5,000 ppm group, one bird was small and a further one was small and thin. In the 10,000 ppm group, a small bird and a very small, thin bird were found, a third bird showed a firm mass at the base of the left kidney.

Validity criteria of the tests can be considered as fulfilled since the mean mortality of control animals was below 10%.

Value used in Risk Assessment	
Value/conclusion	4,488 mg/kg (5d-LC50, *Colinnus virginianus*)
Justification for the value/ conclusion	The value was determined in a valid guideline study performed under GLP conditions with analytical monitoring of the test concentrations. The application type is realistic and therefore reliable for effect assessment. The effects were adequately assessed on the basis of several parameters which are all relevant for the health of the test organisms.

A.4.2.7 Primary and secondary poisoning

Primary poisoning

Data waiving	
Information requirement	Not specified in BPR Annex II
Justification	According to the use patterns of Bronopol (PT 2, 11 and 12), primary poisoning is unlikely and the assessment therefore considered as non-relevant.

Secondary poisoning

Data waiving	
Information requirement	Bioconcentration (Annex II, Title 1, Point 9.1.4 and 9.6) and Bioaccumulation Annex II, Title 1, Point 9.7)

Justification	Bronopol is unlike to bioaccumulate in aquatic or terrestrial environment according to the TGD (2003): It has a low log Kow (-0.42 at 20 °C), it is not highly adsorptive, it does not belong to a class of substances known to have a potential to accumulate in living organisms and has a short degradation half-life of <2 h in the water/sediment test. No further assessment of secondary exposure via the food chain is therefore considered necessary.

A.4.3. Derivation of PNECs

Table 90: Derivation of PNECs

Compartment	PNEC	Remarks/Justification
Freshwater	PNEC(freshwater): 0.00048 mg a.s./L	Organism: Green algae Endpoint: EC10 (72 h) = 0.0048 mg/L (geometric mean concentration) Assessment factor: 10 Extrapolation method: assessment factor Justification: Since the three taxonomic groups (fish, invertebrates, algae) are covered and long-term toxicity data are available for fish and invertebrates, an assessment factor of 10 is applied.
Freshwater for TNM	0.0045 mg a.s./L	Organism: Green algae Endpoint: EC10 (72 h) > 4.5 mg/L Assessment factor: 1000 Extrapolation method: assessment factor Justification: only acute data for the three taxonomic groups (fish, invertebrates, algae)
Sediment	PNEC(sediment): 0.0018 mg a.s./kg ww 0.00826 mg a.s./kg dwt	Organism: not applicable Endpoint: no experimental data available Assessment factor: not applicable Extrapolation method: EPM Justification: Bronopol is not suspected to partition to sediment due to its intrinsic properties. Nevertheless, the PNECsediment can be calculated using the equilibrium method, *i.e.* applying the following formula: $PNEC_{sediment} = (K_{susp\text{-}water} / RHO_{susp}) \times PNEC_{water} \times 1000$ where $K_{susp\text{-}water} = F_{water\ susp} + F_{solid\ susp} \times (Kp_{susp} /1000) \times RHO_{solid}$ being: $F_{water\ susp} = 0.9$ $F_{solid\ susp} = 0.1$ $RHO_{solid} /= 2500\ kg/m^3$ $Kp_{susp} = Foc_{susp} \times Koc = 0.1 * 136$ L/kg (key study geomean value) = 13.6 $K_{susp\text{-}water} = 0.9 + 0.1 \times (13.6/1000) \times 2500 = 4.3$ $PNEC_{sediment} = (4.3/1150) \times 0.00048 \times 1000 = 0.0018$ mg a.s./kgwwt
STP	PNEC(STP): 0.43 mg a.s./L	Organism: Activated sludge Endpoint: EC50 (2.5 h) = 43 mg/L (nominal concentration) Assessment factor: 100 Extrapolation method: assessment factor Justification: Only results from one study type available.
Soil	PNEC(soil): 0.21 mg a.s./kg dw 0.184 mg a.s./kg ww	Organism: native microflora in soil Endpoint: EC10 (28d) = 10.4 mg/kg d.w. (nominal concentration) Assessment factor: 50 Extrapolation method: assessment factor

		Justification: Test results are available for microorganisms, plants and earthworms. The NOEC/EC10 from the test on inhibition to microbial activity can be used as long-term result. According to Guidance on BPR: Vol. IV Part B+C, an assessment factor of 50 can be applied in this case, as there are NOECs for long-term toxicity tests of two trophic levels. Conversion factor to dry weight: the default wet-dry weight conversion factor for soil of 1.13 can be obtained using the default values of RHOsoil=1700 [kgwwt·m-3], RHOsolid=2500 [kg·m-3] and the Fsolid 0.6 [m3·m-3]. Hence, PNEC in ww = PNEC in dw / 1.13. This PNEC is a nominal value, therefore, it should be compared to an initial PEC.
2-BNE		In case a risk assessment is needed for this transient product, for a direct discharge to surface water, PNEC freshwater and sediment for Bronopol can also be used for this metabolite.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.4.4. Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

A.4.4.1 Acute aquatic hazard

Table 91: Summary of key information on acute/ short-term aquatic toxicity relevant for aquatic acute classification

Method	Species	Test material	Results	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 TG 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					

A.4.4.2 Long-term aquatic hazard (including information on bioaccumulation and degradation)

Table 92: 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 TG 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 TG 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					

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 (2-BNE, whose concentration remained constant in equilibrium with the parent) if formed. Other degradation pathways lead to the possible formation of 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 TG 301:

- Modified OECD TG 301B test ([redacted]): 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]): 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]). 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 products are toxic to the environment and fulfil the criteria for classification as hazardous to the aquatic environment (2-BNE and TNM). Hence, Bronopol must be considered as not rapidly degradable for classification purposes.

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, 2-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.

A.4.4.3 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

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

Conclusion on classification and labelling for hazardous to the ozone layer

Based on its chemical and physical properties, the intended use pattern and the concomitant negligible exposure to air, Bronopol is not considered to be hazardous to the ozone layer.

A.6 Assessment of endocrine disruption

A.6.1. SUMMARY

This summary gives an overview of the available data to assess a potential endocrine disrupting potential including the conclusions that could be drawn for Bronopol.

Bronopol has been extensively studied, with a database containing information described in or relevant for all levels (1-5) of the OECD Conceptual Framework for Endocrine Disrupters (2012) [OECD CF 2012].

Level 1 refers to existing data and non-test information, among others Physical & chemical properties, all available (eco)toxicological data from standardised or non-standardised tests, read-across and category approaches. Additionally, studies include QSAR models and other *in silico* approaches, and ADME model predictions refers to existing data.

The *in silico* tools implemented in the Danish QSAR Database, the Endocrine Disruptome and the Estrogen Receptor binding model implemented in the OECD QSAR Toolbox have been assessed to evaluate the computational prediction of the potential of Bronopol to interact with relevant receptors. Overall, these tools consistently provide no evidence of any endocrine activity of Bronopol.

Level 2 studies are *in vitro* assays informing on selected endocrine mechanism(s)/pathway(s). Bronopol has been assessed in a series of *in vitro* assays, including investigations of estrogen and androgen receptor binding, estrogen, androgen, and thyroid receptor transactivation, and steroidogenesis. Information was derived from the ToxCast and EDSP21 databases as well as publicly available literature. Overall, these *in vitro* assays do not indicate any endocrine activity of Bronopol, as supported by the negative predictions of the level 1 models.

Level 3 studies are *in vivo* assays informing on an endocrine mechanism. No mechanistic *in vivo* studies on Bronopol were available in the open literature. Two *in vivo* mechanistic studies were carried out to assess any endocrine activity in non-target organisms other than mammals: Fish Short-term reproduction assay (FSTRA) (OECD TG 299) and Xenopus Eleutheroembryonic Thyroid Assay (XETA) (OECD TG 248).

Level 4 studies are *in vivo* repeated dose toxicity studies, including subacute, subchronic, chronic and developmental toxicity studies in mammals, providing information on adverse effects on endocrine relevant endpoints. Changes in organ weights and histopathology were generally secondary to systemic toxicity, *e.g.* reduced body weight, and were without histologic correlate. Evidence of target organ toxicity found in kidney due to significant histopathological changes and impact on kidney weight in rats in several studies Further, no pattern of effects was observed across available studies. No further treatment-related changes were observed in any organ in the available studies. Overall, these studies do not provide consistent evidence for an endocrine disruptive potential of Bronopol.

Level 5 studies include multi-generation toxicity studies. The available two-generation reproductive toxicity studies showed no treatment-related effect of Bronopol in absence of marked systemic toxicity. The information derived from these studies were evaluated together with available level 4 studies in a weight of evidence approach.

To conclude, the EATS-mediated parameters have been sufficiently investigated with regard to humans and mammals as non-target organisms (section A.6.8.1.2) and with regard to non-target organisms other than mammals upon release into the environment (section A.6.8.2.1). The available mammalian toxicity studies demonstrate that the principal target organ of Bronopol is the kidney, wherein adversity is not considered to be mediated by an endocrine mode of action. No pattern of Bronopol-related adverse effects in endocrine-sensitive organs or endpoints was identified in available OECD Framework Level 4 and 5 *in vivo* toxicity studies. No causal or mechanistic link could be established between Bronopol and EATS-specific effects, and the available OECD Framework Level 1 and 2 *in silico* and *in vitro* data did not provide evidence of an endocrine mode of action.

Conclusively, as the available animal studies do not provide consistent evidence for any

EATS-related adversity which may be linked to an endocrine activity, the substance Bronopol does not meet the ED criteria with regard to humans and mammals as non-target organisms.

With regard to non-target organisms other than mammals, the *in vivo* mechanistic studies carried out in fish and eleutheroembryos of *X. laevis* do not provide a conclusive information about the possible endocrine disrupting properties of Bronopol.

An e-consultation to the ED EG was launched to clarify the validity of the assay performed with eleutheroembryos of *X. laevis*, (OECD TG 248) due to the deviations in the assay conditions and the obtained results. Some experts provided comments about such validity (the study was not considered valid by some experts because of test concentrations that may not be high enough for the response of the XETA) and agreed that the assay could be used just as supportive information additionally, and they suggested a request for further information to clarify the T-modality in non-target organisms other than mammals.

The ENV WG-III-2023 also considered performing an AMA assay to assess the T-modality in non-target organisms other than mammals. A comprehensive and exhaustive review of the available information, jointly with the recommendations received, does not allow the ENV WG to consider that this T-modality is sufficiently investigated.

Additionally, ENV WG-III-2023 agreed on the possibility of discussing the possible need to perform an OECD TG 229 assay with higher concentrations, to evaluate the EAS modalities in non-target organisms other than mammals, once the results of AMA assay are available.

It is possible to conclude that Bronopol is sufficiently investigated, and no adversity based on "EATS-mediated" parameters were found, thus the general conclusion is: Bronopol does not meet ED criteria there is no "EATS-mediated" adversity with regard to humans and mammals as non-target organisms. This is Scenario 1a included in Table 5 of ECHA/EFSA Guidance. However, it cannot be concluded the ED properties for non-target organisms other than mammals, and further information must be requested. This is the Scenario 2a (iii) included in Table 5 of ECHA/EFSA Guidance.

A.6.2. INTRODUCTION

To assess a potential concern for endocrine disrupting effects induced by 2-bromo-2-nitro-1,3-propanediol (Bronopol, CAS No. 52-51-7), all available data comprising experimental data generated by the applicants as well as publicly available data were summarized and presented according to the propositions of the OECD Conceptual Framework for Endocrine Disrupters (2012) [CF 2012] (Table 1).

The CF lists the OECD Test Guidelines and standardized test methods available, under development or proposed that can be used to evaluate chemicals for endocrine disruption. It is intended to provide a guide to the tests available which can provide information for endocrine disrupters' assessment but is not intended to be a testing strategy. The CF organizes information into levels (Level 1-5) increasing in biological complexity and aids the interpretation of results.

The CF has also been implemented in the current guidance for biocides (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, 07 June 2018; further referred to as ECHA/EFSA, 2018).

To receive a comprehensive overview of the available data in the public domain an updated literature research was performed using the following databases: MEDLINE, EMBASE, ESBIOBASE, AGRICOLA, BIOSIS, CABA, CAPLUS, CAS REGISTRY, FSTA, GEOREF, TOXCENTER, PQSCITECH, SCISEARCH, and ANABST. Furthermore, a targeted literature search with focus on ecotoxicological studies has been conducted according to Appendix F of ECHA/EFSA, 2018, in the US National Library of Medicine of the National Institutes of Health (Pubmed) and the National Agricultural Library of the US Department of Agriculture (Agricola) (further described in Annex II).

Table 93 shows all available data for Bronopol for each OECD test method, or other appropriate assays, for evaluating chemicals for endocrine disruption as provided in the CF.

Study results are presented in an overview form only, because this assessment is considered an *addendum* to the biocidal active substance dossier for Bronopol under the Biocidal Product Regulation EU/528/2012 where the detailed study information is already available.

In the subsequent sections of this document (sections A.6.3 to A.6.8), the available data are discussed and finally a conclusion considering the current biocide criteria for endocrine disrupters implemented in the European Biocidal Products delegated Regulation (EU/2017/2100) is given (A.6.9)

Table 93: Listing of all available data (company generated and public domain) for Bronopol according to OECD (2018) Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption

TEST	OUTCOME	SCORE[9]	REFERENCE
Level 1 Existing Data and Non-Test Information			
Physical state at 20°C and 1013 hPa	solid (visual determination)	1	[redacted] 2000 (A3.01.1_02), [redacted] 2001 (A3_1_1-01)
Freezing point at 1013 hPa	129 °C (decomposition at ca. 170 °C)	1	[redacted] 2002 (A3.01.1_01)
Boiling point at 1013 hPa	The normal boiling temperature cannot be determined. At pressures above 60 hPa temperatures decreased at constant pressures as a consequence of thermically caused changes in the test item.	1	[redacted] 2002a (A3.01.2_01)
Density at 20°C	1.894 g/ml at 20 °C	1	[redacted] 2001 (A3.1.1_01)
Vapour pressure at 20°C	$5.1x10^{-3}$ at 20 °C, $1x10^{-2}$ Pa at 25 °C	1	[redacted] 2001 (A3.01.3_01)
Log K_{ow} at 23°C at pH 7	-0.42 (at 20 °C and pH 3–4)	1	[redacted] 2007d (A3_9-02)
Water solubility at 20°C	304 g/L (at pH 5; 20 °C) The determination of the water solubility at pH 7 and above is not reasonable. The test substance is not stable under these conditions.	1	[redacted] 2021b (no BPD-ID)
Viscosity at 20°C	Not applicable, not a liquid		
Flammability	Bronopol is not considered highly flammable; Bronopol does not evolve flammable gases in contact with water; No self-heating was detected up to 400 °C; Bronopol is not pyrophoric; Bronopol is not a readily combustible solid, no spontaneous combustion.	1	[redacted] 2007 (A3.10_01) [redacted] 2006 (A3_11-02) [redacted] 2000 (A3_11-01)

[9] According to Klimisch, Andreae, and Tillmann, Regulatory Toxicology and Pharmacology, **25**:1–5, 1997, with justification given in the accompanying text.

Oxidising properties	Bronopol is not considered an oxidising substance	1	[redacted] 2007 (A3.10_01)
Toxicokinetics	No bioaccumulation potential, based on the low partition coefficient of -0.42 at pH 3-4, the BCFs ≤1 for aquatic and terrestrial ecosystems (QSAR)	1	[redacted] 2007d (A3_9-02)
Biodegradation	Not readily biodegradable	2	[redacted] 2022a (A7.1.1.2.1_02)
All available (eco)toxicity data from standard and non-standard tests	Please refer to dossier		Joint active substance dossier
QSAR predictions	*Danish QSAR Database* Estrogen Receptor α Binding, Full training set (Human *in vitro*): **negative** (SciQSAR) Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*): **negative** (Leadscope and SciQSAR) Androgen Receptor Antagonism (Human *in vitro*): **negative** (CASE Ultra, Leadscope and SciQSAR) Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*): **negative** (Leadscope) Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*): **negative** (Leadscope) *Endocrine Disruptome* Androgen receptor agonist: -4.5, **low probability** Androgen receptor antagonist: -4.4, **low/intermediate probability** Estrogen receptor α agonist: -3.9, **low probability** Estrogen receptor α antagonist: -3.9, **low probability** Estrogen receptor β agonist: -5.0, **low probability** Estrogen receptor β antagonist: -4.2, **low probability** Glucocorticoid receptor agonist: -4.1, **low probability** Glucocorticoid receptor antagonist: -3.8, **low probability** Liver X receptor α: -4.1, **low probability** Liver X receptor β: -4.1, **low probability** Peroxisome proliferator-activated receptor α: -4.2, **low probability** Peroxisome proliferator-activated receptor β: -4.3, **low probability** Peroxisome proliferator-activated receptor γ: -3.7, **low probability** Retinoid X receptor α: -4.5, **low probability** Thyroid hormone receptor α: -4.4, **low probability** Thyroid hormone receptor β: -4.3, **low probability** *OECD QSAR toolbox (v4.2)* Estrogen Receptor (ER) binding of parent compound, transformants and metabolites (v2.2):	2	Danish QSAR Database Endocrine Disruptome[10] OECD QSAR Toolbox OASIS TIMES COMPARA (EPA ED SP21) CERAPP (EPA ED SP21) DEREK Nexus (QSAR prediction reports are included in Annex III)

[10] Likeliness of receptor interactions predicted as low to high probability; prediction can be accessed: *http://endocrinedisruptome.ki.si/docking/fxpvcecxwt/*

	negative *OASIS TIMES report* ER +/-S9 or AR binding or aromatase inhibition: **negative** Aromatase inhibition: **negative** *COMPARA (EPA ED SP21)* ER binding and transactivation: **negative** *CERAPP (EPA ED SP21)* AR binding and transactivation: **negative** *DEREK Nexus* Knowledge-based prediction of potential 5-alpha reductase inhibition, adrenal gland toxicity, androgen receptor (AR) modulation, estrogen receptor (ER) modulation, estrogenicity or thyroid toxicity: **no alert**		
Level 2 *In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) (mammalian and non-mammalian methods)			
Estrogen, androgen and thyroid receptor binding affinity	ER binding assay: **No estrogenic effects** ERα and ERβ binding: **Negative** ToxCast ER Bioactivity Model: **Negative** (score: 0)	2 2 1	[redacted] *et al.* 2007 [redacted] *et al.* 2019 US EPA, ToxCast ER prediction model
Estrogen receptor activity	YES assay (+/- metabolic activation (S9)) **No estrogenic effects** **Negative** (agonistic and antagonistic) *Estrogen/anti-estrogen activity:* ATG_ERa_TRANS_up: **no effect**	2 2 1	[redacted] *et al.* 2007 [redacted] *et al.* 2019 US EPA, ToxCast, in part reported in [redacted] *et al.* 2014

	ATG_ERE_CIS_up: **no effect** Tox21_ERa_BLA_Agonist_ratio: **no effect** Tox21_ERa_LUC_BG1_Agonist: **no effect** Tox21_ERa_BLA_Antagonist_ratio: **no effect** Tox21_ERa_LUC_BG1_Antagonist: **no effect** OT_ER_ERaERa_0480: **no effect** OT_ER_ERaERa_1440: **no effect** OT_ER_ERaERb_0480: **no effect** OT_ER_ERaERb_1440: **no effect** OT_ER_ERbERb_0480: **no effect** OT_ER_ERbERb_1440: **no effect** OT_ERa_EREGFP_0120: **no effect** OT_ERa_EREGFP_0480: **no effect** ACEA_T47D_80 h: **no effect** (80hr_Negative: active; 80hr_Positive: inactive)		
Androgen receptor activity	AR-EcoScreen™ cell line (OECD TG 458): **No agonistic or antagonistic effects** 22Rv1/MMTV cell line ("me-too test" for androgen receptor agonistic/antagonistic effects, OECD project No. 4.99): **No agonistic or antagonistic effects** **Negative** *Androgen/anti-androgen activity* ATG_AR_TRANS_up: **no effect** OT_AR_ARELUC_AG_1440: **no effect** OT_AR_ARSRC1_0480: **change (only highest conc above baseline and active above cytotoxic limit)** OT_AR_ARSRC1_0960: **change (above cytotoxic limit)** Tox21_AR_BLA_Agonist_ratio: **change (only highest conc above baseline and active above cytotoxic limit)** Tox21_AR_BLA_Antagonist_ratio: **change (above cytotoxic limit)** Tox21_AR_LUC_MDAKB2_Agonist: **no effect** Tox21_AR_LUC_MDAKB2_Antagonist: **no effect** TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881: **no effect** TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881: **change (only highest conc above baseline and active above cytotoxic limit)** UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist: **change (only highest conc above baseline and active above cytotoxic limit)**	2 2 1	[redacted] *et al.* 2018 [redacted] *et al.* 2005 US EPA, ToxCast

	UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist: **change (only highest conc above baseline and active above cytotoxic limit)**		
Thyroid activity	*Thyroid -relevant receptors* *Thyroid hormone receptor (TR)* Tox21_TR_LUC_GH3_Agonist: **no effect** Tox21_TR_LUC_GH3_Antagonist: **change (above cytotoxic limit)** *Thyroid-stimulating hormone (TSHR)* TOX21_TSHR_HTRF_Agonist_ratio: **change (above cytotoxic limit)** TOX21_TSHR_HTRF_Antagonist_ratio: **no effect** TOX21_TSHR_HTRF_wt_ratio: **no effect** *Thyrotrophin-releasing hormone receptor (TRHR)* TOX21_TRHR_HEK293_Agonist: **no effect** TOX21_TRHR_HEK293_Antagonist: **no effect**	1	US EPA, ToxCast
	Indicators of hepatic catabolism TOX21_AhR_LUC_Agonist: **no effect** ATG_CAR_TRANS_dn: **no effect** ATG_CAR_TRANS_up: **no effect** TOX21_CAR_Antagonist: **no effect** TOX21_CAR_Agonist: **no effect** ATG_PXRE_CIS_dn: **no effect** ATG_PXRE_CIS_up: **no effect** ATG_PXR_TRANS_dn: **no effect** ATG_PXR_TRANS_up: **no effect** TOX21_PXR_Agonist: **no effect**		
	Deiodinase inhibition (DIO) NHEERL_MED_hDIO1_dn **no effect** NHEERL_MED_hDIO2_dn **no effect** NHEERL_MED_hDIO3_dn **no effect**	1	[redacted] *et al.* 2019 [redacted] *et al.* 2018
	Sodium iodide symporter (NIS) NIS_RAIU_inhibition: **no effect**	2	[redacted] *et al.* 2019
	Amplex UltraRed-thyroperoxidase (AUR-TPO) assay **No TPO activity**	2	[redacted] *et al.* 2016/ US EPA
	Interacion with TH plasma binding proteins TOX21_TRHR_HEK293_Agonist: **no effect** TOX21_TRHR_HEK293_Antagonist: **no effect**		
Steroidogenesis *in vitro*	Extended H295R assay for 10 hormones:	2	[redacted] *et al.* 2016

	Negative *Steroidogenesis in vitro* TOX21_Aromatase_Inhibition: **change (above cytotoxic limit)**	1	US EPA, ToxCast
Other assays	PR-CALUX negative anti-PR-CALUX negative sub-cytotoxic concentrations	2	[redacted] & [redacted] 2018
	Progresterone receptor in Ishikawa cells anti-progestogenic active	2	[redacted] *et al.*, 2015
	GR-CALUX negative anti-GR-CALUX negative sub-cytotoxic concentrationsbinding	2	[redacted] & [redacted] 2018
	Glucocorticoid receptor in Ishikawa cells **anti-progestogenic active**	2	[redacted] *et al.*, 2015
	PPARg2-CALUX negative anti-PPAR g2-CALUX negative sub-cytotoxic concentrations	2	[redacted] & [redacted] 2018
Level 3 *In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s)			
Fish Short-Term Reproduction Assay (FSTRA, OECD TG 229)	*Pimephales promelas*, tested at 0.110, 0.330 and 1.00 mg/L Bronopol did not alter P. promelas reproduction or affect endpoints associated with endocrine activity related to the HPG axis in *P. promelas* and thus, did not cause any adverse effects related to the endocrine system and reproductive performance of fathead minnows NOEC: 1 mg/L **No EAS-specific effects**	3	[redacted] 2022 (A7.4.3.5.3_02)
Xenopus Eleutheroembryo Thyroid Assay (XETA, OECD 248)	Transgenic *Xenopus laevis* eleutheroembryos of the THb/ZIP-GFP transgenic line, tested at three concentrations in spiked and unspiked mode: 16.7, 5.56, 1.85 and 0.617 mg/L Bronopol did not show and induction greater than 12% or a decrease in fluorescence greater than 12%, so Bronopol was found to be thyroid inactive in the XETA. Observed mortality not considered to be test item-related. **No T-specific effects**	3	[redacted] 2022 (A7.4.3.5.3_01)

Level 4 *In vivo* assays providing data on adverse effects on endocrine relevant endpoints			
Repeated dose 21/28-day study (OECD TG 410)	Rabbit, dermal; 0.2% and 0.5%, 21 days exposure Some irritation effects; all other parameters inconspicuous **No EATS-specific effects**	2	[redacted] 1973 (A6.03.2_01)
Repeated dose 28-day study in non-rodents	Dog, drinking water, 0.005, 0.025, 0.05% (4.47, 20.73, 40.59 mg/kg/day in males and 4.27, 15.40, 32.65 mg/kg/day in females), 28 days exposure NOEL (systemic): 0.05% NOEL (local): Not established, due to local irritation of the nasal mucosa **No EATS-specific effects**	1	[redacted] 2006 (A6_03_1-2)
Repeated dose 90-day study (OECD TG 408)	Rat, gavage; 0, 20, 80, 160 mg/kg bw/d (males/females), 90 days exposure NOAEL: <20 mg/kg bw/d High dose (160 mg/kg bw/d): mortality: LD_{100} reached Mid dose (80 mg/kg bw/d): mortality: LD_{50} almost reached; rel. organ weight↑ (thyroid, thymus, adrenals, brain, males; liver, ovaries, females) not related to increase in abs. organ weight, but related to decrease in body weight; abs. organ weight↑ (testes, ovaries); no histopathological correlates; all changes within historical control range; distended renal tubules with infiltrates in adjacent interstitial tissue (1 male) and corticomedullary junction (1 male) Low dose (20 mg/kg bw/d): rel. organ weight↑ (liver, thyroid, male); liver not related to increase in abs. organ weight; abs. organ weight↑ (thyroid, male); no histopathological correlates; all changes within historical control range; no dose-response relationship; distended renal tubules with infiltrates in adjacent interstitial tissue (1 male) and corticomedullary junction (1 male) **No EATS-specific effects**	2	[redacted] 1973 (A6.04.1_01)
	Rat, drinking water, 0.025, 0.075, 0.15% (21.82, 59.16, 124.71 mg/kg/day for males and 28.18, 78.19, 136.31 mg/kg/day for females), 90 days exposure NOAEL: 0.075% (59.16 mg/kg/day) in males; 0.15% (136.31 mg/kg/day) in females NOEL: not established for males due to reduced water consumption at all dose levels,	2	[redacted] 2006 (A6_04_1-2)

	0.025% for females **No EATS-specific effects**		
	Rat, drinking water, 60, 250 and 1,000 ppm (6.2, 24.3, 83.9 mg/kg/day in males and 6.8, 25.5, 86.0 mg/kg/day in females) Increased T3 in 1000 ppm recovery group (13 week treatment followed by 4 week recovery) males (no change in T3, T4, or TSH in males or females of any dose level at the end of treatment) NOAEL: 250 ppm (24.3/25.5 mg/kg/day in males/females) **No EATS-specific effects; increased T3 was observed only in recovery males at 4 weeks after cessation of exposure and was considered sporadic, since the differences were only slight and individual control values ranged all below the 2s limits of historical controls**	1	[redacted] 2001 (A6_04_1-1)
	Dog, gavage; 0, 4, 8, 20 mg/kg bw/d (males/females), 90 days exposure NOAEL: 8 mg/kg bw/d High dose (20 mg/kg bw/d): vomiting, WBC-count↓ but within normal limits; rel. organ weight↑ (liver, spleen due to 1 male), abs. organ weight ↑ (spleen; due to 1 male); all other parameters inconspicuous Mid dose (8 mg/kg bw/d) and low dose (4 mg/kg bw/d): no treatment-related effects **No EATS-specific effects**	2	[redacted] 1973 (A6.04.1_02)
	Dog, drinking water, 0.005, 0.025, 0.05% (3.76, 15.04, 28.39 mg/kg/day in males and 3.76, 18.75, 32.16 mg/kg/day in females) NOEL: ≥0.05% (28.39/32.16 mg/kg/day in males/females) **No EATS-specific effects**	1	[redacted] 2007 (A6_04_1-3)
One-generation reproduction toxicity study (OECD TG 415)	Rat, gavage; 0, 20, 40 mg/kg bw/d (males/females), 19 weeks exposure NOAEL (parental): 20 mg/kg bw/d NOEL (reproduction): 40 mg/kg bw/d	2	[redacted] 1973 (A6.08.2_02)

	High dose (40 mg/kg bw/d): parental animals body weight change↓ (male); Mean pup and litter weights at birth and at day 4 post parturition were slightly below control values. Those differences were without any statistical significance (p>0.05) and at weaning, litter weight turned back to control values. All other parameters were inconspicuous. Low dose (20 mg/kg bw/d): no treatment-related findings **No EATS-specific effects**		
Prenatal developmental toxicity study (OECD TG 414)	Rabbit, gavage, range-finder; 0, 1, 3, 10, 20, 40, 80 (GD7-13)/160 (GD14-16) mg/kg bw/d (females), exposure on GD 7-19 NOAEL maternal toxicity: 40 mg/kg bw/d; NOEL developmental toxicity: 40 mg/kg bw/d High dose (80/160 mg/kg bw/d): maternal effects: mortality↑, food consumption↓, size and quantity of faecal pellets↓, body weight and body weight gain↓, pregnancy and implantation data inconspicuous; terminated at GD17: necropsy revealed haemorrhage and ulceration of gastric mucosa 1, 3, 10, 20, or 40 mg/kg bw/d: no treatment-related effects **No EATS-specific effects**	2	[redacted] 1991b (A6.08.1_04)
	Rabbit, gavage; 0, 5, 20, 40, 80 mg/kg bw/d (females), exposure on GD 7-19 NOAEL maternal toxicity 40 mg/kg bw/d; NOAEL developmental toxicity 40 mg/kg bw/d High dose (80 mg/kg bw/d): maternal effects: food consumption↓, size and quantity of faecal pellets↓, body weight and body weight gain GD 7-11↓, pregnancy and implantation data inconspicuous; teratogenic effects: foetal weight↓, runted foetuses↑, retarded ossification↑, non-ossification of the fore- and hind limb epiphyses↑ 5, 20, or 40 mg/kg bw/d: no treatment-related effects **No EATS-specific effects**	1	[redacted] 1991a (A6.08.1_01)
	Rabbit, gavage, 3, 10, 30 mg/kg/day NOEL (maternal): 10 mg/kg/day NOEL (fetal): 10 mg/kg/day	1	[redacted] 2007 (A6_08_1-1)

	No EATS-specific effects; fetal axial skeletal malformations only in presence of maternal toxicity		
	Rat, gavage, range-finder; phase I: 0, 3, 10, 30, 100; phase II: 60, 80, 100 mg/kg bw/d (females), exposure on GD 6-15 NOAEL maternal toxicity: 10 mg/kg bw/d; NOEL developmental toxicity: 100 mg/kg bw/d High dose (100 mg/kg bw/d): maternal effects: mortality↑, food consumption↓, body weight and body weight gain↓, necropsy of sacrificed animals revealed ulceration of gastric mucosa 80 mg/kg bw/d: body weight gain↓ (GD 6-9) 60 mg/kg bw/d: body weight gain↓ (GD 6-9) 30 mg/kg bw/d: body weight gain↓ (GD 6-9) 3, or 10 mg/kg bw/d: no treatment-related effects No treatment-related effects on reproduction parameters **No EATS-specific effects**	2	[redacted] 1993 (A6.08.1_05)
	Rat, gavage; 0, 10, 28, 80 mg/kg bw/d (females), exposure on GD 6-15 NOAEL maternal toxicity: 80 mg/kg bw/d; NOEL developmental toxicity: 80 mg/kg bw/d High dose (80 mg/kg bw/d): maternal effects: body weight gain↓ (GD6-7), pregnancy and implantation data inconspicuous; no adverse effect of treatment on embryonic and foetal development 10, or 28 mg/kg bw/d: no treatment-related effects **No EATS-specific effects**	1	[redacted] 1995 (A6.08.1_02)
Chronic toxicity and carcinogenicity studies (OECD TG 451-3)	Rat, drinking water; 10, 40, 160 mg/kg bw/day corresponding to 7, 32 and 142 mg/kg bw/day (re-estimation based on further stability testing) (males/females), 104 weeks exposure NOAEL: 10 mg/kg bw/d	2	[redacted] 1976 (A6.07_01_a) (reported in [redacted] *et al.* 1978 (A6_05-01) and US EPA RED 1995 (A6_05-02))

	High dose (160mg/kg bw/d): mortality↑, food consumption↓, body weight gain↓, grooming activity↓, rel. kidney weight↑, but without histopathological correlate; ulceration of epithelium in the stomach↑, occasional papillomata, dilatation of sinusoids in gastric lymph nodes↑ due to irritancy of Bronopol Mid dose (40 mg/kg bw/d): food consumption↓, body weight gain↓ Low dose (10 mg/kg bw/d): no treatment-related findings **No EATS-specific effects**		
	Mouse, dermal, non-occlusive; 0, 0.2%, 0.5% (males/females), 80 weeks exposure NOAEL 0.2% High dose (0.5%): body weight gain↓ (males, w26-52), hair-loss around treatment area↑ (3w); skin effects (3 females, 1 male) due to irritancy of Bronopol Low dose (0.2%): no treatment-related findings **No EATS-specific effects**	2	[redacted] 1975 (A6.07_02_a) (reported in [redacted] *et al.* 1978 (A6_05-01) and US EPA RED 1995 (A6_05-02))
Fish early life stage (ELS) toxicity test (OECD TG 210)	*Oncorhynchus mykiss*, 0, 2.25, 4.41, 6.85, 11.5, 21.5, 40 mg/L, (nominal). Measured: 0.44, 0.07, 0.29, 1.18, 1.94 and 7.48 mg/L)exposed for 49 days NOEC 21.5 mg/L nominal. 1.94 mg/L (measured) (mortality↑ >NOEC). No sub-lethal adverse effects on development **No EATS-specific effects**	2	[redacted] 1996 (A7.4.3.2_01)
Fish, Juvenile Growth Test (OECD TG 215)	*Oncorhynchus mykiss,* 0, 0.32, 1.0, 3.2, 10, 32 mg/L (nominal, measured (only the three highest concentrations were analysed): 2.6, 9.3 30 mg/L), exposed for 28 days NOEC 2.6 mg/L (mortality↑ >NOEC) No sub-lethal adverse effects on development, no statistically significant change in body wet weight and pseudo-specific growth rate for wet weight. No EATS-specific effects	1	[redacted] 2007 (A7_4_3_1-01)
Daphnia reproduction test	*Daphnia magna*, 0, 0.017, 0.053, 0.17, 0.53, 1.7 mg/L, exposed for 21 days	2	[redacted] 1992 (A7.4.3.4_01)

(OECD TG 211)	NOEC 0.27 mg/L (immobility↑ >NOEC) Bronopol and its breakdown products caused a significant increase in mortality of 58% among the early life stages of trout at the highest nominal concentration tested which was 4Omg/l. A small increase in mortality was seen at 21.5 mg/l, at 16%, this was not significant when compared with the control group. The mortality in the control group was 11%. No significant differences on final weights, lengths an condition indices between any of the groups exposed and the controls. No other sub-lethal adverse effects were seen. LC50=39 mg/L nominal concentration. No sub-lethal adverse effects on reproduction No presence of males and ephippia were refered.		
	Daphnia magna, 0, 0.025, 0.05, 0.1, 0.2, 0.4 mg/L (nominal, measured: 0.0044, 0.013, 0.029, 0.060 and 0.110 mg./L), exposed for 21 days NOEC 0.06 mg/L No sub-lethal adverse effects on development All daphnia exposed to 1.7 mg/l were recorded dead within 4 days of exposure. At the other concentrations, immobility (including mortality) was less than 20% at the end of the 21-day exposure period; this was not significant when compared to the control. Hence, the EC50-value for 21 days of exposure was between 0.53 and 1.7 mg/l (nominal). No significant (>10%) immobilization (including mortality) among newborn was observed at concentrations of 0.53 mg/l (nominal) or lower. No biologically significant differences in numbers of unhatched eggs were observed between the concentrations tested during the reproduction phase. There was no adverse effect on reproduction at any of the concentrations that had been maintained throughout the 21-day exposure period.	2	[redacted] 2004 (A7_4_3_4-01)
Other test data	Peri- and postnatal development: Rat, gavage; 0, 20, 40 mg/kg bw/d (females), exposure on GD 15 until day 21 post parturition NOAEL maternal toxicity: 40 mg/kg bw/d; NOEL developmental toxicity: 40 mg/kg bw/d NOEL postnatal development: 40 mg/kg bw/d	2	[redacted] 1973 (A6.08.1_03)

	Neither the dams nor the pups showed treatment-related effects at the dose levels tested in the present study **No EATS-specific effects**		
	Xenopus tadpoles exposed to a mixture of 23 chemicals (including Bronopol) associated with unconventional oil and gas operations for 3 weeks, concentration range: 0.1, 1, 5 and 10 µg/L Mixture appeared toxic to tadpoles at doses of 5 and 10 µg/L Exposure induced acute alterations of immune function and antiviral immunity Results cannot be attributed solely to the exposure to Bronopol as a complex mixture was used as test substance; no conclusion on adverse effects on endocrine relevant endpoints induced by Bronopol can be drawn. **inconclusive**	2	[redacted] *et al.* 2018
	Exposure of pregnant mice with a mixture of 23 chemicals; no conclusions can be drawn for Bronopol as complex mixtures were used as test substance **inconclusive**	2	[redacted] *et al.* 2016; [redacted] *et al.* 2015
Level 5 *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism			
Two-Generation reproduction toxicity study (OECD TG 416)	Rat, drinking water, 0.01, 0.05, 0.15% (F0 males: 8.8, 41, 104 mg/kg/day; F0 females: 11.5-14.2, 53.2-67.8, 129-183 mg/kg/day; F1 males: 10.5, 52.6, 141 mg/kg/day; F1 females: 11.9-14.8, 58.3-67.1, 156-187 mg/kg/day) NOEL (systemic): 0.01% (8.8-14.8 mg/kg/day) NOEL (reproductive): 0.05% (41-67.8 mg/kg/day) <u>F0</u> Decreased absolute adrenal weight in 0.05% males, considered spurious due to lack of dose response relationship, value within the historical control range, and absence of treatment-related histopathologic changes in the adrenals of high dose males Increased thyroid weight in 0.15% females, within historical control range Increased incidence of very slight follicle dilatation of the thyroid, without appreciable hyperplasia/hypertrophy or differences in the colloidal staining properties in 0.05 and 0.15%	1	[redacted] 2008 (A6_08_2-1)

	females Vacuolization of scattered individual hepatocytes (fatty change) in 0.15% females 2 cases of dystocia in 0.15% dams <u>F1</u> Decreased absolute brain weight in F1 females and F2 weanlings at 0.15%, considered spurious based upon values within historical control range and decreased body weight Vacuolization of scattered individual hepatocytes (fatty change) in 0.15% females Increased post-implantation loss (18.8 versus 6.8% in control) in 0.15% dams with associated decreases in gestation survival and stillborn pups 2 total litter losses in 0.15% dams **Increased thyroid weight and histopathological changes were observed only in F0 females (histopathology of thyroid not performed on F1 females due to absence of change in thyroid weight) thus interpreted as equivocal; reproductive effects were attributable to 6- 0.15% dams, several of which exhibited clear maternal toxicity in late gestations, thus not considered endocrine-mediated**		
	Rat, drinking water, range-finder; 0, 25, 50, 100, 200 mg/kg bw/d (males/females), 5-6 weeks exposure (14 days pre-mating, ca.21 days of gestation, 3 days post parturition) NOAEL parental toxicity: <25 mg/kg bw/d; NOEL reproduction toxicity: 200 mg/kg bw/d NOEL offspring: 200 mg/kg bw/d High dose (200 mg/kg bw/d): parental effects: 1 male sacrificed moribund, necropsy rev. ulcers and thickened mucosa in the non-glandular stomach, as well as bloody gastrointestinal contents, food consumption↓ (males/females), w1), water consumption↓ (males/females), body weight gain↓(males/females) 100 mg/kg bw/d: body weight gain↓ (males; females only until w3), water consumption↓ (males; females only until w2) 50 mg/kg bw/d: body weight gain↓ (males; females only until w3), water consumption↓ (males; females only until w2) 25 mg/kg bw/d: body weight gain↓ (males; females only until w3), water consumption↓ (males; females only until w2) Copulatory interval, fertility indices and duration of gestation were not affected by the	1	[redacted] 1986 (A6.08.2_03)

	treatment. None of the considered litter parameters showed treatment-related effects and the pups showed no treatment-related abnormalities. **No EATS-specific effects**		
	Rat, drinking water; 0, 25, 70, 200 mg/kg bw/d (males/females) Exposure: F0: from 80 days prior mating (mating duration 15d) until end of the weaning period of the F1 young; F1: from F1 weaning until weaning of the F2 young and thereafter for 33 to 47 days. NOAEL (F0, F1) systemic toxicity: 25 mg/kg bw/d; NOAEL (F0, F1) reproduction toxicity: 70 mg/kg bw/d NOAEL offspring: 200 mg/kg bw/d <u>High dose (200 mg/kg bw/d):</u> F0 parental effects: kidney weight↑ (females), food consumption↓ (males/females, w1+2), water consumption↓ (males/females), body weight, body weight gain↓ (males /females), kidneys with granular appearance (2 males; 4 females), incidence nephropathy↑ (females) F1 parental effects: body weight, liver weight, body weight gain↓ (males/ females) food consumption↓ (males/females), water consumption↓ (males/females), kidneys with granular appearance (1 male; 2 females), incidence nephropathy↑ (females) F2b effects: abs. liver weight↓ (males/females), abs. kidney weight↓ (females) Reproduction effects: F1a, F1b: fertility index slightly↓ (females), pup mean body weight slightly↓ (males/females); F2a, F2b: pup mean body weight slightly↓ (males/females); some abnormalities (*e.g.* pup size↓ related to body weights↓) resulting rather from high systemic toxicity than indicating reproductive toxicity <u>Mid dose (70 mg/kg bw/d):</u> F0 parental effects: food consumption↓ (males), water consumption↓ (males/females) F1 parental effects: water consumption↓ (males/females) <u>Low dose (25 mg/kg bw/d):</u> F0 parental effects: food consumption↓ (males), water consumption↓ (males/females) F1 parental effects: water consumption↓ (males/females) **No EATS-specific effects**	1	[redacted] 1987 (A6.08.2_01_a)

A.6.3. LEVEL 1

Level 1 comprises existing data and non-test information, covering physical & chemical properties, (eco)toxicological data from standardized or non-standardized tests, and read across, chemical categories, QSARs and other *in silico* predictions, and ADME model predictions.

The physical-chemical properties of Bronopol are shown in Table 93. In addition, for the purpose of this assessment of potential endocrine disruptive properties of Bronopol, the *in silico* predictions of the following computational tools has been evaluated (see also Table 93) (Annex II). A literature research was also performed (Annex III).

A.6.3.1. *In silico* predictions

A.6.3.1.1. *Danish QSAR Database*

Using the Danish QSAR Database, predictions for Bronopol could be generated with regard of potential interactions with the estrogen receptor, the androgen receptor as well as thyroperoxidase.

No binding capacity towards the estrogen receptor α was predicted via Leadscope and SciQSAR. Furthermore, absence of any antagonistic capacity of Bronopol towards the androgen receptor is consistently predicted by CASE Ultra, Leadscope and SciQSAR. While Bronopol was outside the applicability domains of the tools assessing the potential binding to the thyroid receptor, the substance was calculated as negative with regard to its potential to inhibit thyroperoxidase activity (Leadscope) [Danish QSAR Database report; Annex II].

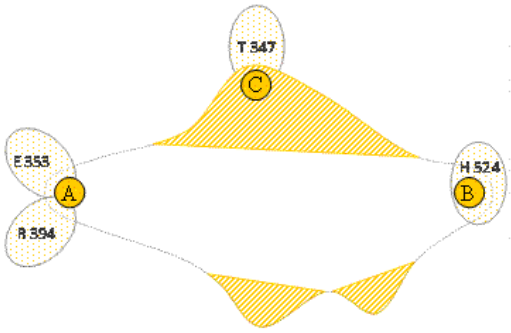
Taken together, these QSAR tools consistently do not give any indication for any endocrine activity of Bronopol.

A.6.3.1.2. *Endocrine disruptome*

The Endocrine disruptome computational tool predicts the endocrine activity of a chemical against 16 structures belonging to 12 nuclear receptors. The calculated likeliness of the specific endocrine activity is assigned to one of 4 categories, *i.e.* low, low/intermediate, intermediate/high or high probability. For the androgen receptor, a low/intermediate probability of an antagonistic activity was estimated, while no agonistic activity was predicted (low probability). For all other receptors assessed, *i.e.* estrogen receptor α and β, glucocorticoid receptor, liver X receptor α and β, peroxisome proliferator-activated receptor α, β and γ, retinoid X receptor α, as well as thyroid hormone receptor α and β, no agonistic and/or antagonistic activity was predicted for Bronopol [Endocrine disruptome report; Annex III].

A.6.3.1.3. *OECD QSAR toolbox v4.2*

No binding capacity towards the estrogen receptor was predicted using the Estrogen Receptor Binding tool (v. 2.2) of the OECD QSAR Toolbox (v. 4.2). Bronopol is predicted as non-binder in this *in silico* tool based on the non-cyclic structure. Estrogen receptor (ER) binding is a molecular initiating event much like protein binding [[redacted] *et al.* 2006] that may lead to a series of adverse outcomes, which are typically linked to reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER-binding and possible subsequent endocrine disruption [[redacted] and [redacted] 2008]. Popular among these are the "four phase" assessment that includes Comparative Molecular Field Analysis (CoMFA) [[redacted] *et al.* 2004] and the Common Reactivity Pattern Approach (COREPA) [[redacted] *et al.* 2003]. Since the ER-binding is a receptor-mediated event, particular organic functional groups, size and shape are critical to binding potency. A schematic representation of an ER binding pocket with its three sites of interaction (A, B, C) is shown in figure below.



Schema of estrogen receptor (ER) binding pocket.

Chemicals, such as Bronopol or its metabolites, that have a molecular weight less than 500, and do not possess a cyclic structure are reported to be non-binders to the ER [[redacted] and [redacted] 2008; [redacted] *et al.* 2004].

In addition, the rtER Expert System was used to estimate estrogen receptor binding. The US EPA profiler consists of molecular definitions mimicking the structural criteria of chemical classes potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES). The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity. No structural alert for estrogen receptor binding was observed. In addition to the parent compound also potential metabolites and transformants of Bronopol were investigated regarding ER binding. None of the predicted metabolites or transformants were predicted as ER binding substances regardless of the metabolism simulator used [OECD QSAR Toolbox; Annex II].

A.6.3.1.4. *OASIS Times v2.27.19.413*

Binding to either estrogen receptor (ER) or androgen receptor (AR) was further estimated *in silico* using three QSAR models implemented in OASIS TIMES (V2.27.19.13): i) binding affinity to androgen receptor (AR) (without metabolisation) and ii) binding to estrogen receptor (ER) with and iii) without S9-metabolic activation). Bronopol or metabolites thereof were in domain of the QSAR models regarding estrogen receptor binding, but out of domain for modeling of androgen receptor binding. However, none of the three models predicted a binding of Bronopol or metabolites thereof to ER (without or with metabolisation of parent compound) or AR. Furthermore, aromatase inhibition was estimated using OASIS TIMES algorithms. Bronopol was out of domain and result of the prediction was that Bronopol does not inhibit aromatase [OASIS Times report; Annex II].

A.6.3.1.5. *ToxCast: Models*

ToxCast Pathway Model (AUC) provides a value (range from 0-1) for Androgen and Estrogen Receptors activity each (in agonist or antagonist mode). If any of these values exceed 0.1 then there is a significant interaction [ToxCast Models report; Annex II].

- Estrogen receptor activity as described in [redacted] *et al.* 2015.
- Androgen receptor activity as described in [redacted] *et al.* 2017

Bronopol was not predicted as active on Estrogen and Androgen Receptors (both agonist and antagonist mode).

CERAPP (Collaborative Estrogen Receptor Activity Prediction Project; as described in [redacted] *et al.* 2016) demonstrated the effectiveness of combining many QSAR models trained on HTS data to prioritize a large chemical list for estrogen receptor activity. The limitations of single models were overcome by combining all models built by the consortium into consensus predictions. Bronopol was predicted to be a non-binder of ER and to be non-capable to agonize or antagonize ER [ToxCast Models report; Annex II].

CoMPARA is a larger scale collaboration between 35 international groups, following the steps of CERAPP to model androgen receptor activity using a common training set of 1746 compounds provided by US EPA. Eleven high-throughput screening ToxCast/Tox21 *in vitro*

assays were integrated into a computational network model to detect true AR activity. A key result is a consensus model of AR agonist and antagonist activity that is run against the DSSTox chemical library. These results are intended to be used in prioritization for compounds for follow-up testing. Bronopol was predicted to be a non-binder of AR and to be non-capable to agonize or antagonize AR [ToxCast Models report; Annex II].

A.6.3.1.6. *3.1.6 DEREK Nexus (V6.0.1, Nexus V2.2.1)*

DEREK Nexus (V6.0.1, Nexus V2.2.1) is an expert, knowledge-based toxicology software which gives predictions for a variety of endpoints. This knowledge-based system did not show an alert for potential endocrine-related endpoints such as 5-alpha reductase inhibition, adrenal gland toxicity, androgen receptor (AR) modulation, estrogen receptor (ER) modulation, estrogenicity or thyroid toxicity [DEREK Nexus report; Annex II].

A.6.3.2. Summary

The available QSAR tools consistently predict no endocrine activity of Bronopol.

A.6.4. LEVEL 2

Level 2 comprises *in vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) (mammalian and non-mammalian methods).

Bronopol was evaluated in several *in vitro* assays on endocrine activity available in published literature. Moreover, a series of *in vitro* high-throughput screening assays are available for Bronopol from the US EPA Toxicity Forecaster (ToxCast) as well as the Endocrine Disruption Screening Program for the 21st Century (EDSP21).

These data were primarily derived from US EPA CompTox Chemicals Dashboard (available at: https://comptox.epa.gov/dashboard/chemical/details/DTXSID8024652.

A.6.4.1. Estrogen receptor activity

Bronopol was evaluated for ER binding *in vitro* using a commercial estrogen receptor binding assay. In this assay, the affinity of Bronopol to human ER-α is measured. Bronopol was tested in concentrations between 0.2 µg/mL and 700 µg/mL. 17β-estradiol served as reference substances. Bronopol showed no binding activity to hERα [[redacted] *et al.* 2007]. In a recent study, Bronopol was evaluated for ER binding *in vitro* using commercial human ER-α and ER-β fluorescence polarization competitive binding assays. Bronopol showed no binding to hERα or hERβ [[redacted] *et al.* 2019].

In the *in vitro* high-throughput screening assays (US EPA, ToxCast) Bronopol was tested for estrogen binding potential, and for androgen, and thyroid receptor binding potential too (see the paragraphs A.6.4.2 and A.6.4.4). These tests were conducted following standardised test protocols and are adequately reported. The data/results and concentrations are reported only graphically and in a tabular overview(Table 1). Bronopol was evaluated for estrogenic activity *in vitro* using a yeast estrogen screen (YES) assay, with and without addition of a rat liver preparation (S9 mix). The assay used yeast cells stably transfected with the human ERα gene and an expression plasmid containing the estrogen response elements (ERE) controlling the reporter gene. Estrogen receptor transactivation was assessed by detection of the reporter gene β-galactosidase with and without metabolic activation (S9 mix). Acute *in vitro* toxicity tests using yeast were conducted to ensure that estrogenic activity was not limited by toxic effects. 17β-estradiol, p-nonylphenol and bisphenol A served as reference substances. Bronopol showed no activity to ERα in presence or absence of metabolic activation [[redacted] *et al.* 2007].

In another reporter transactivation assay according to OECD TG 455 Bronopol was evaluated along with other 134 chemicals for estrogenic activity using stably transfected VM7Luc4E2 cells. After incubation for 20–24 h, visual scoring for cell viability was conducted for all experiments before luminescence measurements. In accordance with the guideline, reference substances (agonist: bisphenol A and spironolactone; antagonist: raloxifene/E2, 2-hydroxytamoxifen and di-n-butylphthalate) were used to determine the proficiency of the

test system. The cytotoxic effects of ER agonists and antagonists, which are detected by comprehensive tests, were determined in test concentration ranges using the CellTiter-Glo luminescent cell viability assay. Although detailed insight into the Bronopol analyses was not documented in the publication, the authors mentioned that Bronopol showed no antagonistic or agonistic activity in the ERTA assay [[redacted] *et al.* 2019].

In the *in vitro* high-throughput screening assays (US EPA, ToxCast21) Bronopol was tested for transactivation of ERα and Erβ, Receptor dimerization and Proliferation. These *in vitro* tests were conducted following standardized test protocols and are adequately reported.

Bronopol was found active in three of the 28 ER available studies: TOX21_ERb_BLA_Agonist_viability, TOX21_ERR_Agonist, TOX21_PGC_ERR_Agonist. In all cases the results were flagged as less than 50% activity, and the last assay is only active at highest concentrations above baseline. Bronopol does not show effects on protein stabilization, regulation of gene expression, regulation of transcription factor activity or cell proliferation.

Part of these results are also documented in [redacted] *et al.* (2014), who reported about some assays of the EDSP21 screening program. Bronopol, along with 1813 other chemicals, was evaluated in a panel of 13 *in vitro* high-throughput screening (HTS) assays. The panel of *in vitro* assays interrogated multiple end points related to estrogen receptor (ER) signalling, namely binding, agonist, antagonist, and cell growth responses. The results from the *in vitro* assays were used to create an ER Interaction Score. Bronopol showed no activity (ER Interaction Score = 0) in all assays.

Further *in vitro* studies on the potential endocrine activity of bronopol using non-standardized test protocols were identified in the open literature. These studies are shortly reported here but excluded from further analysis due to the use of non-standardized tests or major deficiencies as described below.

[redacted] and [redacted] (2018) refered evaluation of bronopol for estrogenic activity using commercial CALUX assays, using a hormone response element linked to luciferase. ERα-CALUX cells were incubated with up to 100 µM of bronopol. Bronopol did not act as receptor agonist up to 100 µM concentration. In the antagonistic mode of the assay, bronopol reduced luciferase activity in the two highest concentrations (50 and 100 µM), however, it was clearly demonstrated that cytotoxicity (EC_{50} 72.5 µM (95%CI: 11.1-474 µM)) was involved in those effects.

[redacted] *et al.* (2014) transfected HepG2 cells with a reporter gene assay, in which cells were transfected with estrogen response element linked to luciferase and cytomegalovirus-β-galactosidase to assess (anti-)estrogenic or (anti-)androgenic activities of bronopol. Bronopol showed no estrogenic activity. In the antagonistic assay a reduction of the positive control was observed at 3.3 µM (IC_{10}) of bronopol. However, cytotoxicity, which could overlay/mimic antagonistic effects, was not measured, thus this study is no indication for antioestrogenic action of bronopol, if at all this equivocal result would be indicative for antagonistic MoA, the potency of bronopol would be 100,000 fold lower than the control.

[redacted] *et al.* (2015) further investigated potential antagonistic effects of bronopol in a different cell-line, an Ishikawa cell-based reporter assay, which was established from an endometrial adenocarcinoma. As those cells have a different metabolic competency, compared to above used HepG2 liver cells, potentially higher amounts of bronopol could have been present. Bronopol was tested in concentrations between 0.1 – 10 µM in 0.1% of methanol. However, results are presented only graphically in a very confusing way, most curve-fits were non-sigmoidal, number of independent replicates was not reported and no EC_{50} could be reached (also true for almost all chemicals tested (n = 24)). Significant cytotoxicity was reported for concentrations ≥10 µM of bronopol. Due to several deficiencies, this publication is considered as non-indicative for anti-estrogenic action of bronopol.

A.6.4.2. Androgen receptor activity

Bronopol was evaluated for AR transactivation *in vitro* applying OECD TG 458 (using AR-EcoScreen™ cell line) and a "me-too test method" (using 22Rv1/MMTV cell line), which was

adopted as OECD project 4.99. Cell lines were stably transfected with an AR-responsive promoter and Bronopol was tested at concentrations in the range of 10^{-1} to 10^{-7} mg/mL. The test systems were validated prior to testing of Bronopol using appropriate controls, such as DHT and mestanolone (agonism) and hydroxyflutamide or bicalutamide and bisphenol A (antagonism), respectively, as positive controls and DEHP as negative control in suitable concentrations. Bronopol was negative as both an AR agonist and antagonist in both test systems [[redacted] *et al.* 2018]. Before OECD TG 458 was established, Bronopol (along with 252 other industrial chemicals) was screened for AR transactivation *in vitro* using AR-EcoScreenTM cells. 10 µM DHT served as agonist positive control and 10 µM hydroxyflutamide as antagonist positive control. Bronopol was negative as both an AR agonist and antagonist [[redacted] *et al.* 2005].

In the *in vitro* high-throughput screening assays (US EPA, ToxCast) Bronopol was tested for agonist/antagonist activity against, and transactivation of human androgen receptor AR. These *in vitro* tests were conducted following standardized test protocols and are adequately reported. The data/results and concentrations are reported only graphically and in a tabular overview.

Bronopol was found to be inactive at sub-cytotoxic concentrations in the *in vitro* screening battery. Activity was found in TOX21_ARE_BLA_Agonist_ch2. Bronopol could act as a regulation of transcription factor.

Additionally, US EPA EDSP tested Bronopol in 14 assays. Besides the above mentioned Bronopol was active for the following assays: OT_AR_ARSRC1_0480 and _0960, and UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist and _Antagonist. Among them, only OT_AR_ARSRC1_0960 is active at concentrations below baseline.

Furthermore, bronopol was evaluated by [redacted] *et al.* (2014) for androgenic activity using a reporter gene assay, in which HepG2 cells were transfected with androgen response element linked to luciferase and cytomegalovirus-β-galactosidase. Overall, this study is considered non-indicative for antiandrogenic action of bronopol.

[redacted] *et al.* (2015) further investigated potential antagonistic effects of bronopol in a different cell-line, an Ishikawa cell-based reporter assay, which was established from an endometrial adenocarcinoma. As those cells have a different metabolic competency, compared to above used HepG2 liver cells, potentially higher amounts of bronopol could have been present of incubation. Bronopol was tested in concentrations between 0.1 – 10 µM in 0.1% of methanol. However, results are presented only graphically in a very confusing way, most curve-fits were non-sigmoidal, number of independent replicates was not reported and no EC_{50} could be reached (also true for almost all chemicals tested (n = 24)). Significant cytotoxicity was reported for concentrations ≥10 µM of bronopol. Due to the mentioned deficiencies, this publication is considered as non-indicative for anti-androgenic action of bronopol.

Additionally, [redacted] and [redacted] (2018) indicate bronopol was evaluated for androgenic activity using commercial CALUX assays. AR-CALUX cells were incubated with up to 100 µM of bronopol. After lysis of the cells receptor activation was determined via a luciferase assay. 5α-dihydrotestosterone (agonist) or flutamide (FLT) (antagonistic) were used as reference substances. Bronopol did not act as receptor agonist up to 100 µM concentration. In the antagonistic assay, bronopol reduced luciferase activity to below 80% of control wells at 100, 50, and 25 µM bronopol, however these concentrations were cytotoxic to AR-CALUX cells according to the MTT assay (EC_{50} 39.4 µM). Sub-cytotoxic concentrations of bronopol did not reduce luciferase activity below approximately 80% of the control condition.

A.6.4.3. Thyroid receptor activity

Related to the thyroid hormone synthesis, the Amplex UltraRed (AUR-TPO) TPO inhibition assay was developed by the US EPA ([redacted] *et al.*, 2016). The screening for potential thyroid disruptors was conducted by assessing the potential for thyroperoxidase (TPO) inhibition. Thyroid microsomes pooled from rat thyroids were used to assess the TPO inhibition at a single concentration (87.5 µM). For bronopol no TPO inhibition was detected (score: 13.5 of 100%; threshold for positive hit response ≥ 20%) in the AUR-TPO assay.

In the *in vitro* high-throughput screening assays (US EPA, ToxCast) Bronopol was tested for agonist/antagonist activity against, and transactivation of thyroid receptor ThR. These *in vitro* tests were conducted following standardised test protocols and are adequately reported. The data/results and concentrations are reported only graphically and in a tabular overview. For the assays related assess the activity (agonism and antagonism) of thyroid hormone receptor (TR) suggested by [redacted] (2001), only the TOX21_TR_LUC_GH3_Antagonist is active.

Additionally, ToxCast21 includes some assay related to the thyroid stimulating hormone receptor (TSHR), TOX21_TSHR_HTRF_Agonist_ch1 and _ratio are active, both involved in regulation of transcription factor activity.

The thrytotrophin-releasing hormone receptor (TRHR) was also included in ToxCast21. None of the assays is active for Bronopol (TOX21_TRHR_HEK293_Agonist and _Antagonist mode).

Furthermore, for the assays listed ([redacted], 2021) as indicators of hepatic catabolism, only one assay is active (ATG_PXRE_CIS_dn), despite that the data coming from the assay must be used with caution, due to the difficulty to detect loss of signal. Additionally, should be considered that not all the substances that activate these receptors and downstream metabolism cause thyroid effects *in vivo*.

Bronopol could have activity on regulation of transcription factor.

[redacted] *et al.* (2015) investigated potential antagonistic effects of bronopol in an Ishikawa cell-based reporter assay, which was established from an endometrial adenocarcinoma. Bronopol was tested in concentrations between 0.1 - 10 µM in 0.1% of methanol. However, results are presented only graphically in a very confusing way, most curve-fits were non-sigmoidal, number of independent replicates was not reported and no EC_{50} could be reached (also true for almost all chemicals tested (n = 24)). Significant cytotoxicity was reported for concentrations ≥10 µM of bronopol. No anti-thyroidogenic activity was observed for bronopol, however, as mentioned above the reliability of that publication was low due to several deficiencies.

Bronopol does not show any effect related to the inhibition of sodium iodide symporter (NIS) NIS_RAIU_inhibition [Wang *et al.* 2019]. Although Br^- is a metabolite that could interfere with the thyroid, the low levels at which it is formed as a metabolite (A6.02_08_a, Glass *et al.* 1993, and A6.02_08_b, Thakrar 1993) and the absence of adversity in the numerous *in vivo* studies allow to assume that the effect of this anion, in the case of bronopol, is not relevant to human health..

Bronopol does not show any effect related to the inhibition of sodium iodide symporter (NIS) NIS_RAIU_inhibition [[redacted] *et al.* 2019]. Although Br^- is a metabolite that could interfere with the thyroid, the low levels at which it is formed as a metabolite (A6.02_08_a, [redacted] *et al.* 1993, and A6.02_08_b, [redacted] 1993) and the absence of adversity in the numerous *in vivo* studies allow to assume that the effect of this anion, in the case of bronopol, is not relevant to human health.

Despite this metabolization in mammals, no Thyroid effects were observed in any of the assays carried out in mammals.

Bronopol does not show any effect on Deiodinase inhibition (DIO) processes (NHEERL_MED_hDIO1_dn, _hDIO2_dn, and _hDIO3_dn) [[redacted] *et al.* 2019, [redacted] *et al.* 2018].

A.6.4.4. Steroid receptor activity

In the *in vitro* high-throughput screening assays (US EPA, ToxCast) Bronopol was tested for steroidogenesis, including the inhibition of aromatase. Bronopol was found to be active with an log AC50 of 1.88 µM. However, activity was only observed at the highest concentrations, well above the cytotoxic limit, with an AC50 of baseline.

No inhibition of aromatase was observed in sub-cytotoxic concentrations of Bronopol. One nonreceptor-mediated endocrine disrupting mechanism is via effects on aromatase, an enzyme involved in steroidogenesis and critical for maintaining the normal *in vivo* balance

of androgens and estrogens. Aromatase (CYP19A1) is a member of cytochrome P450 isozymes involved in the steroid biosynthesis pathway. In the last step of the steroid biosynthesis pathway, aromatase converts testosterone (T) to estradiol (E2) or androstenedione to estrone. Aromatase plays a key role in maintaining the androgen/estrogen balance in many tissues throughout the body. Abnormal levels of aromatase enzyme activity have been linked to endocrine-related diseases.

In addition, Bronopol was tested in a series of further *in vitro* models, addressing various cytochrome P450 isozymes. Only a few certain CYP enzymes play a key role in the steroid hormone biosynthesis [[redacted] and [redacted] 2011], whereas most of the CYP enzymes tested with Bronopol are involved in the steroid metabolism (if at all steroid related), and may be only secondary relevant for steroidogenesis, but are included for informative purposes. Bronopol was found inactive in the vast majority of these assays (27/32: LTEA_HepaRG_CYP1A1_dn, _CYP1A2_dn and _up, _CYP24A1_1_dn and _up, _CYP2B6_dn and _up, _CYP2C19_dn and _up, _CYP2C8_dn and _up, _CYP2C9_dn and _up, _CYP2E1_dn and _up, _CYP3A4_dn and _up, _CYP3A5_dn and _up, _CYP3A7_dn and _up, _CYP4A11_dn and _up, _CYP4A22_dn and _up, _CYP7A1_dn and _CYP7A1) and active in 4 cell-free systems (NVS_ADME_hCYP1A1, _hCYP1A2, _hCYP2C19, and _hCYP2C9) and in LTEA_HepaRG_CYP1A1_up, reported with and AC50 of 68.85 µM, with only the highest concentration above the baseline and well above the cytotoxic limit of 9.058 µM.

Furthermore, results of the above-mentioned *in vitro* high-throughput screening approach could not be confirmed in a guideline-conform study including aromatase-sensitive hormones. The OECD validated H295R steroidogenesis assay (OECD TG 456) was adapted to a high-throughput format by [redacted] *et al.* (2016) and a novel HPLC-MS/MS approach was used to successfully quantify 10 hormone analytes in parallel: a) glucocorticoids: 11-deoxycortisol, deoxycorticosterone, cortisol, b) progestagens: 17α-hydroxyprogesterone, 17α-hydroxypregnenolone, progesterone, c) androgens: androstendione, testosterone; d) oestrogens: oestrone, oestradiol. The assay evaluated 13 steroid hormones (four glucocorticoid hormones, four Progestogen hormones, 3 Androgen hormones, and 2 Estrogen hormones). Some modification from the OECD guideline had to be introduced, *e.g.* duplicate instead of triplicate measurements or a lower cell viability (70% instead of 80%) or different statistics. Chemicals of the ToxCast chemical libraries I, II and partly III were used in a screening approach at its respective maximal tolerated concentration (100 µM). An absolute fold change cut-off of ≥1.5 (relative to DMSO controls on a per-plate basis) was used to identify chemicals eliciting effects on hormone levels. Bronopol did not alter any hormone levels under the experimental conditions. The screening tests were conducted to standardised test protocols and are adequately reported. The data and results are reported in a tabular overview. None of the CEETOX_H259R assays tested was active for Bronopol.

A.6.4.5. Other assays

In another high-throughput analysis focusing on phases I and II of the US EPA's ToxCast project 331 cell-free enzymatic and ligand-binding high-throughput screening (HTS) assays were used to analyse 976 chemicals. Half maximal activity concentrations (AC50) were identified for 729 chemicals in 256 assays. Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). In that HTS *in vitro* screening Bronopol showed – according to the ranking nomenclature of the US EPA's EDSP21 program – weak interactions (comparable to non-specific interactions) with some cytochrome P450 enzymes (CYP2C19, CYP2C9, CYP1A2) and neurokinin 2 receptor and very weak interactions (AC50 in the upper µM level) with some other enzymes and receptors. The assays are adequately reported [[redacted] *et al.* 2013].

A.6.4.6. Summary

The potential endocrine activity of bronopol has very extensively been assessed in various *in vitro* assays. The publicly available data indicate some endocrine activity of bronopol.

A.6.5. LEVEL 3

Level 3 data comprises *in vivo* assays providing data about selected endocrine mechanism(s) / pathway(s).

Bronopol was evaluated in a mechanistic *in vivo* study. No further information on mechanistic *in vivo* assays for Bronopol could be identified in the public literature.

A.6.5.1. Fish Short-Term Reproduction Assay (FSTRA, OECD TG 229)

In the Fish Short-Term Reproduction Assay (FSTRA, OECD TG 229), [redacted 2022].96 fathead minnows (Pimephales promelas) were exposed to three test concentrations – 1.00, 0.330 and 0.110 mg/L Bronopol – and a dilution water control over a 21d exposure period. The exposure concentrations were held constant via a flow-through system with a media flow rate of 2.7 L/h and a media exchange rate of 6.5 tank volumes per day. No effect on any of the following parameters was observed:

- Survival: All fish survived during the study
- Behaviour and appearance: Clinical signs of toxicity were not observed during the conduct of the present study.
- Fecundity: Mean fecundity measured in the Bronopol treatments on SD 21 was not significantly increasing relative to the control (Jonckheere-Terpstra test, p=0.25).
- Secondary sex characteristics: Mean fecundity measured in the Bronopol treatments on SD 21 was not significantly increasing relative to the control (Jonckheere-Terpstra test, p=0.25).
- Vitellogenin: Mean plasma VTG measured in the Bronopol treatments in female fish were not significantly different from control (Dunnett's test, p=0.2953, 0.5546, and 0.14 for the 0.110 mg/L, 0.330 mg/L, and 1.00 mg/L Bronopol treatments, respectively). Mean plasma VTG measured in the Bronopol treatments in male fish were not significantly increasing relative to the control, respectively (Jonckheere-Terpstra test, p=0.4356).
- Evaluation of gonadal histopathology: no treatment-related findings in the testes of male fish exposed to Bronopol. Mean testicular stage scores in control versus treated fish were comparable.

  Some granulomatuous inflammations were found in females (minimal to moderate) was greater in the ovaries of females exposed to 0.110, 0.330, or 1.00 mg/L Bronopol as compared to those of control females.

  Additionally, the prevalence of increased oocyte atresia (minimal and moderate) was substantially greater in the ovaries of females exposed to 0.330 mg/L Bronopol as compared to control females. However, the prevalence of increased atresia in 1.00 mg/L group females was identical to that of controls (*i.e.*, there was no monotonic dose-response pattern), post-ovulatory follicles (indicative of recent spawning) were abundant in 0.330 mg/L group females, and fecundity was not reduced in that group; therefore, the increased atresia in the 0.330 mg/L group was considered to be a spurious occurrence unrelated to treatment. It is also important to recognize that in fractionally-spawning fish such as fathead minnows, the morphologic appearance of the ovary (or testis) primarily reflects gonadal activity during the several day period prior to sacrifice, as opposed to the entire 21-day study period. Additionally, the frequency of post-ovulatory follicles at a given time point is influenced by the ovarian stage (*i.e.*, phase of breeding cycle). Mean ovarian stage scores were generally comparable among control females and those exposed to Bronopol.
- Liver and kidney histopathology: The treatment-related microscopic findings which occurred in the liver and biliary system were not clinically appreciable, as there were no treatment-related impacts on body weight or feeding behaviour and therefore these off-target effects were not considered endocrine-mediated.

Considering the Acceptance Criteria stated in OECD TG 229 § 13, the test should be considered valid since the validity criteria are met.

- Test concentration ±20% of mean measured concentration: met.

- Control mortality ≤10% in any replicate of the control: met.

- Pre-exposure Spawing-Spawing in each replicate tank prior to initiation of chemical exposure: met.

- DO ≥ 60% of saturation (4.9 mg/L): met.

- Water temperature 25 ± 2 ºC (± 1.5 °C between test vessels at any one time during the exposure period): met.

However, it must be considered that the validity criteria are met does not not necessarily mean that the study is relevant and reliable. The combination of reliable and relevant makes the study useful for hazard assessment. The correct test concentrations setting is an essential part of the toxicity test and should be considered as a critical factor for the relevance of the test. The study is not reliable enough due to the low test concentrations used during the assay (top dose 1.0 a.s. mg/L). The top dose was applied after a range finding test, (10, 1.0, 0.1, 0.01 mg/L and control). 10 mg/L produced a mortality of 18.5%>10% mentioned for the MTC in the guideline. Since no adverse effects were observed at 1.0 mg/L that was the top dose selected.

Taking into account those low tested concentrations and the deviations from the Guideline, the results obtained must be considered inconclusive.

Assuming all the considerations aforementioned, a clear decision cannot be taken. A WoE has been done, using this assay just as supportive information, related to the not alteration of reproduction or affected endpoints associated with endocrine activity related to the HPG axis of the tested fish species (fathead minnow, *P. promelas*), and assuming that further evidence/information could be needed to reach a clearer assessment. Bearing in mind the unclear results, a WoE approach was proposed.

A.6.5.2. *Xenopus* Eleutheroembryonic Thyroid Assay (XETA, OECD TG 248)

Based on the information obtained from the mammalian studies which indicate that no effects related to the thyroid mode of action, it is possible consider that Bronopol meets the Case 1 as suggested in Annex to: Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311, "Use of the XETA in the assessment strategy of the ECHA/EFSA Guidance". In this case, the thyroid modality is considered sufficiently investigated for mammals and no effects were observed. That allows request a XETA (OECD TG 248), instead of an AMA (OECD TG 231). Then the T-modality could be considered sifiently investigated for non-target organisms other than mammals.

The Xenopus EleutheroembryonicEleutheroembryo Thyroid Assay (XETA, OECD TG 248) [[redacted] 2022]was performed over three individual runs, each lasting 72 hours. Since full mortality was observed in the highest test concentration of the first run (50 mg/L Bronopol), the concentration range was adapted for the next two runs by adding 0.617 mg/L as lowest test concentration to the remaining three test concentrations from run 1 (16.7, 5.56 and 1.85 mg/L Bronopol). All test concentrations were tested in the presence ("spiked mode") and absence ("unspiked mode") of a co-treatment with 3.25 µg/L of T3 (Triiodothyronine) and together with three different control solutions (test medium control, T3 control and T4 (Thyroxine) control as saturation control). The test solutions were renewed twice daily to ensure a constant exposure. The endpoint of the XETA is induction of fluorescence in eleutheroembryos. When transcription of the genetic construct is activated or inhibited following chemical exposure, eleutheroembryos express more or less GFP (green fluorescent protein) and, therefore, emit more or less fluorescence, compared to unexposed individuals where fluorescence remains at the basal level. A positive result in the XETA is given if at least one concentration tested, including the highest, is active in T3-spiked and/or unspiked mode. In T3-spiked mode, an active concentration is defined as a concentration giving a statistically significant fluorescence increase or decrease of at least 12 % compared to the T3 control. In the unspiked mode, a concentration is considered to be active only if a statistically significant fluorescence increase of at least 12 % compared to the test medium control is observed.

None of the tested Bronopol concentrations showed a statistically significant change of at least 12% in fluorescence compared to the respective control [2022].

Due to the deviations in the assay from the OECD guidance, some doubts arose about the validity of the assay:

- Tested concentrations: it is not clear enough the tested concentrations, even though the renewal of the tested solutions is reduced (12 h semi-static) due to the rapid degradation of Bronopol. Despite the calculations (overall average), the concentrations do not remain within 20% of the nominal concentration in a 12 h time frame; but it is especially relevant for those concentrations measured at 12 h when in some cases the measured concentration does not reach the 50% of the nominal.
- After the e-consultation with the Endocrine Disruptors Expert Group, the experts considered that the concentrations may be high enough for the response of the XETA. It is still unknown whether or not the XETA is thyroid active at concentrations above 12.5 mg/L.
- The temperature reached 26.7°C, which could have relevance to the fate of Bronopol and the concentration at which the Eleutheroembryos are exposed. That could be consistent with the lowest concentration of Test Substance in run 3 when a temperature deviation occurred in the last 12 hours.

For that reason an e-consultation to the Endocrine Disruption Expert Group was launched in February 2023. Comments were received from the experts from Netherland, France and Germany.

The assay can be considered as valid since all validity criteria are fulfilled. However, and as it is mentioned in the paragraph A.6.5.1 the fact that the validity criteria are met does not necessarily mean that the study is relevant and reliable. The combination of reliable and relevant makes the study useful for hazard assessment. The correct test concentration setting is an essential part of the toxicity test and should be considered as a critical factor for the relevance of the test. All experts agreed on the non-conclusive results obtained, mainly because those low test concentrations may not be high enough for the response of the XETA. The test does not provide a clear conclusion regarding the question of T-mediated activity since the individual runs show conflicting results regarding the observed induction of fluorescence (*i.e.* a significant effect (>12%) on fluorescence in the highest concentration in run 1 in the non-spiked exposure group, a significant reduction (>12%) of fluorescence in run 2 and a significant increase (>12%) of fluorescence in run 3 – both in the T3-spiked exposure groups). The substance showed thyroid inactive at the measured test concentrations ≤ 12.5 mg/L. However, it is still unknown whether or not the XETA is thyroid active at concentrations above 12.5 mg/L. The results of the assay cannot be considered conclusive, mainly due to the very low concentrations tested (1.85, 5.56, 16.7 mg/L), that may not be high enough to obtain any response of the XETA assay.

However, the experts did not consider relevant the increased temperature during the assay.

Additionally, some concerns were exposed related to the Modes of Action covered by the XETA assay. Specially related to the Inhibition od sodium iodide symporter (NIS) due to the possible effects of Br^- ions which are known to be NIS competitive inhibitors, generated during the metabolization of Bronopol. That mode of action is unlikely due to the information about the inactivity on NIS_RAIU_inhibition assay (*et al.* 2019), accesible via the US EPA Dashboard, where a dose-response curve is available. .

Others MoAs not covered by XETA (Thyroid peroxidase-TPO- Inhibitor, Modulators of TH transport via interaction with TH plasma binding proteins) but it seems that these MoAs are unlikely due to the inactivity in Amplex UltraRed-thyroperoxidase (AUR-TPO) assay [*et al.* 2016/ US EPA] and Interacion with TH plasma binding proteins (TOX21_TRHR_HEK293_Agonist, TOX21_TRHR_HEK293_Antagonist), accessible via US EPA Dashboard, where a dose-response curve is available. *et al.* 2016 is a screening assay that uses a cut-off inhibition value. Bronopol has a lower value, that could imply a negative result.

For the reasons explained above the XETA could be considered as an adequate option to assess the T-modality in non-target organisms, but due to the deviations of the assay provided by the applicants, the results could be used as supportive information jointly with the rest of information related to thyroid. The use of these results in a WoE approach is considered inappropriate by the WG.

The inconclusive results on T-modality make necessary to request further assays (AMA; OECD TG 231), as agreed in WGIII2023, and suggested by ED EG.

A.6.6. LEVEL 4

Level 4 data comprises *in vivo* assays providing data on adverse effects on endocrine relevant endpoints.

A.6.6.1. Repeated dose 28-day studies

A.6.6.1.1. Repeated dose 28-day oral toxicity study in dogs

Bronopol was administered to groups of 2 male and 2 female Beagle dogs in the drinking water for at least 28 days. Dose levels of 0.005, 0.025, and 0.05% in water corresponded to dose levels of approximately 4.47, 20.73, and 40.59 mg/kg bw/day in males and 4.27, 15.40, and 32.65 mg/kg bw/day in females. Control animals received the vehicle, municipal drinking water adjusted to pH 4. Animals were observed for mortality, morbidity, and clinical observations, including neurobehavioral findings. Water consumption, ophthalmoscopic examinations, clinical pathology, organ weights, and select tissue histopathology was assessed. No evidence of systemic toxicity was observed. The only effects considered related to treatment were multifocal hypertrophy of mucous cells in the stomach of 1 male and 1 female given 0.05% bronopol and multifocal, subacute to chronic inflammation of the nasal mucosa in both males and 1 female of each dose level. These responses were attributed to local irritation following contact with the test material. Based on the local irritation and inflammation of the nasal mucosa at all dose levels, a NOEL was not established. The NOEL for systemic toxicity was 0.05% bronopol in drinking water. ([redacted], 2006)

A.6.6.1.2. Repeated dose 21/28-day dermal toxicity study (OECD TG 410)

Bronopol (purity unspecified), suspended at 0.2% and 0.5% in 2.5% methyl cellulose was applied to the abraded skin of rabbits (5 animals/sex/dose), at a dose level of 1 mL/kg body weight, daily, 7 days a week, for a period of 3 weeks. At the end of the daily 6 h exposure period the skin was washed and blotted dry. Observations and examinations included: behavioral changes and signs of toxicity, local skin reaction, body weight gain, food consumption and ophthalmologic examinations. Prior test initiation and at termination, blood samples were collected for analysis of a series of hematological and clinical-chemical parameters (packed cell volume, hemoglobin, red cell count, total white cell count and differential count, plasma urea, total serum proteins, serum alkaline phosphatase and serum glutamic-pyruvic transaminase). At the end of the treatment period, the animals were subjected to gross pathology, organ weighing and histopathology (brain, pituitary, thyroid, thymus, salivary glands, stomach, liver, pancreas, kidneys, adrenals, duodenum, spleen, heart, ileum, lungs, gonads, uterus, urinary bladder, gall bladder, lymph nodes, marrow smear, mid-colon, skin (untreated and treated) and eyes). No clinical effects were observed, and no mortality was reported. Some irritation effects on the skin were observed in both dose groups, but all other examined parameters were inconspicuous (reliability 2). ([redacted] *et al.*, 1973; US EPA RED, 1995)

A.6.6.2. Repeated dose 90-day studies

Five 90-day drinking water or gavage toxicity studies were performed with bronopol, 3 in rats (OECD TG 408) and 2 in dogs (OECD TG 409).

A.6.6.2.1. Repeated dose 90-day oral toxicity study in rodents 1 (OECD TG 408)

Groups of 8 male and 8 female Crl:CD(SD) rats were given drinking water solutions containing 0, 0.025%, 0.075%, or 0.15% bronopol for 90 days in order to establish dose levels for a subsequent two-generation reproductive toxicity study. These values corresponded to time-weighted average concentrations of 0, 22, 59, or 125 mg/kg/day for males and 0, 28, 78, or 137 mg/kg/day for females. Parameters evaluated were daily cage-side observations, weekly clinical observations, body weights, feed consumption, water consumption, hematology, prothrombin time, clinical chemistry, urinalysis, selected organ weights, and gross and histopathologic examinations. Water consumption was reduced in males from all treatment groups and in females given 0.075% and 0.15% bronopol. This effect was attributed to decreased palatability of bronopol in drinking water. The reduction in water consumption correlated with slight decreases in feed consumption and body weight in the high dose males only. Histologic examination revealed a slight nephropathy in 3 of 8 high-dose males, variably characterized by slight bilateral multifocal tubular epithelial degeneration with regeneration, bilateral multifocal dilatation of medullary tubules, slight subacute to chronic multifocal interstitial inflammation, thickening of the tubular and glomerular basement membranes, and interstitial fibrosis. Accompanying these histologic changes were increases (approximately 10%) in relative kidney weights. The high-dose males had a statistically significant decrease in serum alanine aminotransferase activity that was considered treatment-related, but not adverse. There were no other treatment-related effects on any other study parameters. The NOEL for female rats was 0.025% but could not be determined in males due to decreased water consumption at all dose levels. The NOAEL was 0.15% for females and 0.075% for males. ([redacted] *et al.*, 2006)

A.6.6.2.2. Repeated dose 90-day oral toxicity study in rodents 2 (OECD TG 408)

Bronopol was administered in the drinking water to groups of 10 male and 10 female Wistar rats using concentrations of 0, 60, 250 and 1,000 ppm for approximately 13 weeks. In parallel, 10 male and 10 female rats per group were treated with the vehicle (acidified water) or 1,000 ppm and observed for reversibility, continuance or delayed occurrence of toxic effects during a recovery period of about 4 weeks. The mean nominal daily bronopol consumption for each treated group for 13 weeks was 6.2, 24.3, and 83.9 mg/kg bw/day in males and 6.8, 25.5, and 86.0 mg/kg bw/day in females. Recover groups receiving 1000 ppm had a mean nominal daily bronopol consumption of 81.8 mg/kg bw/day for males and 85.8 mg/kg bw/day for females. Parameters evaluated included mortality, clinical signs, a functional observational battery (FOB), body weight, feed consumption, water consumption, ophthalmological examinations, hematology, clinical chemistry, urinalysis, organ weights, and gross and histopathological examination. Water consumption was markedly reduced in animals receiving concentrations of 1,000 ppm. Hematologic investigations revealed a decrease in hemoglobin concentrations at term of treatment in male rats receiving 1,000 ppm. Differential blood count showed no toxicologically relevant differences between dose groups and controls. At end of treatment quantitative urinalyses showed low urine volumes and high osmolality in male and, to a lower extent, in female animals receiving 1,000 ppm. Relative kidney weights were statistically significantly increased in female rats receiving 250 ppm and above. Male rats revealed a tendency towards higher relative kidney weights at 1,000 ppm. Histopathology showed minimal to slight changes in the kidneys of both genders receiving 1,000 ppm. They comprised an increased incidence of basophilic tubules (females), hyaline casts at the corticomedullary junction (males) and in the loops of Henle at the renal papilla (both genders). In two males dosed at 1,000 ppm, the cortical tubules appeared to be minimally dilated. Except for the hyaline casts in the loops of Henle, all findings were reversible within the recovery period. A statistically significantly higher T3 level was identified in high dose recovery males. This was considered due to chance since the differences were only slight and individual control values ranged all below the 2s limits of historical controls. Further, no difference was observed in T3, T4, or TSH levels in males or females of any treatment group and no histopathologic correlate was observed. The NOAEL was 250 ppm (24.3 mg/kg bw/day for males and 25.5 mg/kg bw/day for females)

based upon morphological effects on the kidney. ([redacted], 2001)

A.6.6.2.3. Repeated dose 90-day oral toxicity study in rodents 3 (OECD TG 408)

Bronopol (> 98%) was administered by gavage daily for 13 weeks to CD rats (M/F; 20 animals per dose each) in doses of 0, 20, 80 or 160 mg/kg bw/d. Distilled water was used as vehicle. Feed and water consumption and body weight were measured throughout the study. The following observations/examinations were included in the study: clinical examinations, mortality, ophthalmoscopy, urinalysis and hematology, organ weights (liver, kidneys, adrenals, testes, uterus, ovaries, thymus, spleen, brain, heart, pituitary and thyroid), gross pathology and histopathology (adrenals, brain, pituitary, thyroid, thymus, esophagus, salivary glands, tongue, stomach, small and large intestines, caecum, ileum, jejunum, liver, kidneys, spleen, heart, trachea, lungs, aorta, gonads, uterus, prostate, seminal vesicles, female mammary gland, urinary bladder, lymph nodes, pancreas, sciatic nerve, femoral marrow, skeletal muscle, skin, and eyes). Animals died or had to be killed *in extremis* in all dose groups, the LD_{100} was reached at the top-dose, at 80 mg/kg bw/d 30-45% of the animals died or had to be killed *in extremis* and at 20 mg/kg bw/d 1 female animal died in week 10 of the treatment. As necropsy revealed no abnormalities, this case of mortality was not considered to be treatment-related. Urinalysis, hematological and clinical-chemical parameters, ophthalmology were unaffected/inconspicuous. Clinical symptoms were respiratory distress, abdominal distension in the mid and high dose group. In addition, bw gain and food consumption were reduced significantly, but returned to control levels in the mid dose group until the second week of treatment. In the low dose group, only one male animal showed respiratory distress and reduced bw gain but recovered until the third week of treatment. At necropsy, only few organs showed significant changes in absolute and/or relative weights; however, these differences were partly or completely related to differences in body weight. Moreover, the liver weights of 20 mg/kg bw/day treated males and the ovaries weights of 80 mg/kg bw/day treated females were significantly different from controls but still were within the normal range for the rat strain used. Gross pathology revealed no treatment-related abnormalities. However, histopathology revealed distended renal tubules containing eosinophilic material in one male of each the 20 and the 80 mg/kg bw/day group; this finding was further accompanied by mononuclear cell infiltration in the adjacent interstitial tissue. In two further animals (again one male of each the 20 and the 80 mg/kg bw/day group) dilated tubules with eosinophilic material within the corticomedullary junction were reported. No such effects were seen in controls, they therefore were considered to be treatment-related. However, as no dose-relationship was evident, these effects were equivocal. The NOAEL under the experimental conditions was below 20 mg/kg bw/d (reliability 2). ([redacted] *et al.*, 1973; US EPA RED, 1995)

A.6.6.2.4. Repeated dose 90-day oral toxicity study in non-rodents 1 (OECD TG 409)

Groups of 4 male and 4 female Beagle dogs were administered bronopol at dose levels of 0.005, 0.025, and 0.05% in water for at least 90 days. These dose levels corresponded to respective dose levels of approximately 3.76, 15.04, and 28.39 mg/kg bw/day in males and 3.76, 18.75, and 32.16 mg/kg bw/day in females. The control group received the vehicle, municipal drinking water adjusted to pH 4. The test material or vehicle was available *ad libitum*. Parameters evaluated were mortality, morbidity, clinical observations including neurobehavioral findings, body weights, food consumption, water consumption, ophthalmoscopic examinations, clinical pathology (hematology, clinical chemistry, urinalysis), organ weights, and gross and histopathological examination. All animals survived until the scheduled termination intervals, and there were no test material related findings associated with any evaluation parameter. The NOEL was considered to be ≥ 0.05% (highest exposure level) bronopol in water. ([redacted], 2007)

A.6.6.2.5. Repeated dose 90-day oral toxicity study in non-rodents 2 (OECD TG 409)

Bronopol (99.2%) was administered by gavage daily (7 days/week) for 13 weeks to Beagle dogs (M/F; 3 animals/dose each) in doses of 0, 4, 8 or 20 mg/kg bw/d. Distilled water was used as vehicle/control. Feed and water consumption and body weight were measured throughout the study. The following observations/examinations were included in the study: clinical examinations, mortality, ophthalmoscopy, urinalysis, clinical chemistry and hematology, organ weights (liver, kidneys, adrenals, prostrate, testes, uterus, ovaries, thymus, spleen, brain, heart, pituitary, lung, pancreas and thyroid), gross pathology and histopathology (adrenals, brain, pituitary, thyroid, thymus, esophagus, salivary glands, tongue, stomach, small and large intestines, caecum, ileum, jejunum, liver, kidneys, spleen, heart, trachea, lungs, aorta, gonads, uterus, prostate, female mammary gland, urinary bladder, gall bladder, lymph nodes, pancreas, peripheral nerve, femoral marrow, skeletal muscle, skin, and eyes). Doses were chosen based on a pre-test with 1 animal/dose and 16-day administration of 0, 20, or 40 mg/kg bw/d (not reported here). Initially, the dogs were first dosed with the test substance and then were fed (*ca*. 1 hour after treatment). Vomiting was mainly observed in the 20 mg/kg bw/day group during the first 6 weeks of treatment; in the remaining treated groups (4 and 8 mg/kg bw/day) single cases of vomiting also were observed during the 6 first weeks of treatment. Because of the increased incidence of vomiting, dosing/feeding routine was changed after the first 6 weeks of treatment and the dogs then first were fed and then were dosed (after *ca*. 2 hours). When dosing/feeding routine was changed (*i.e.* after week 6) and the dogs were fed prior to dosing, incidence of vomiting clearly was reduced. No mortality occurred, bw gain, food and water consumption, ophthalmology were inconspicuous. After 6 weeks of treatment, no treatment-related changes in hematological or clinical-chemical parameters were observed, but the urine samples from two females of the 4 mg/kg bw/day group and one female of the 20 mg/kg bw/day group contained blood pigments and red blood cells. After 12 weeks of treatment, a statistically significant decrease in mean total white cell counts was reported for the 8 mg/kg bw/day and the 20 mg/kg bw/day groups when compared to control; however, the findings remained within normal limits. Clinical-chemical parameter and urinalysis were inconspicuous for all groups. At necropsy, the relative liver weight of the 20 mg/kg bw group was significantly increased when compared to control; this was mainly due to one male of this group (relative liver weight for this animal: 4.03%, *i.e.* upper normal limit). The liver weights for the remaining treated groups were within normal limits. The mean absolute and relative spleen weights for the 20 mg/kg bw group were found to be significantly above the control value. This effect was related to one male of this group (spleen weight 95.2 g vs. 43 – 56 g for control males). The spleen weights for the remaining treated groups were within normal limits. Neither gross pathological nor histopathological treatment-related changes in bronopol-treated dogs of both sexes were seen. The NOAEL under the experimental conditions was 8 mg/kg bw/d (reliability 2). ([redacted] *et al.*, 1973; US EPA RED, 1995)

A.6.6.3. Combined Chronic toxicity/Carcinogenicity studies (OECD TG 451-3)

A.6.6.3.1. Chronic toxicity and carcinogenicity study in rats 1

Sprague-Dawley rats (45/sex/dose) were administered bronopol (purity or a.i. content: > 99.7%) in acidified (pH 4) drinking water for 104 weeks in a chronic feeding/carcinogenicity study. Doses were 0, 10, 40 or 160 mg/kg/day (0, 7, 32 and 142 mg/kg bw/d after reevaluation; Groups 1, 2, 3, 4), corresponding to group mean values of 0, 10.5, 40.2 or 152.2 mg/kg/day (males) and 0, 10.4, 40.7 or 158.4 mg/kg/day (females). Besides a dose-related decrease of water intake due to the unpalatability of bronopol in all dose groups, treatment-related effects were observed only in Groups 3 and 4. The following observations/ examinations were included in the study: body weight, food and drinking water consumption, food efficiency, ophthalmoscopy, clinical signs and mortality, urinalysis, hematology and blood chemistry, gross pathology at necropsy, organ weights (adrenal, testis, ovary, uterus, seminal vesicles, prostate, pituitary, thyroid, heart, lung, kidney, spleen, liver, and brain) and histopathology (all abnormal tissues, brain, pituitary, thymus,

salivary glands, stomach, caecum, ileum, liver, kidneys, spleen, heart, lungs, gonads, uterus, adrenals, urinary bladder, lymph nodes, pancreas, bone marrow, eye, blood smears). At the end of treatment blood and urine samples were drawn for hematology and plasma chemistry analyses and urinalysis, respectively. In Group 3 (40 mg/kg bw/day), treatment-related effects included lower food intake (7%) during weeks 53-78 for the males, reduced weight gain (20-52%) during weeks 27-78 for the males, and squamous metaplasia, inflammation or atrophic acini in the salivary glands of 12/25 (48%) males and 3/23 (13%) females. In Group 4 (160 mg/kg bw/day), treatment-related statistically significant effects included reduced grooming activity in both sexes during the second year of dosing, high mortality in the males (80%) and the females (62%); decreased weight gain during weeks 3-78 among males (13-84%) and during weeks 7-78 among females (11-53%), weight loss during weeks 78-104 among males and females, lower food intake among males (9-16%) during weeks 13-104. Effects on organ weights were decreased absolute weights of heart (29%), liver (35%), lungs (12%), seminal vesicles (47%), testes (20%) and thyroid (26%), all in high dose males, increased relative weights of adrenals, brain, kidneys, liver and lungs in high dose males and females and increased relative weights of pituitary in high dose males. Histopathological effects in high dose animals included stomach lesions in 20/54 (37%) males and 15/52 (29%) females, whereas only 1/56 (1.8%) control males and 1/59 (1.7%) control females had these lesions, an increased incidence of progressive glomerulonephrosis in males (48 vs. 28% in controls) and females (38 vs. 7.7% in controls), sinusoid dilatation in the gastric lymph node in 33 males and 23% females (none in controls), and squamous metaplasia, dilatation of the ducts, acinar atrophy and/or inflammation of the salivary glands in 12/13 (92%) males and 11/20 (55%) females. Because of a significant decrease in water consumption (32-53% (males) or 24-40% (females), the urine output was also reduced in high dose animals (10-46% for males and 31-40% for females). No such changes were seen in the control and the low and mid doses groups. At the lowest tested dose of 10 mg/kg bw/day, no conspicuous differences from control could be evidenced, and the only clear effect, which was reported, consisted of a slight decrease in water uptake and was due to a palatability problem. Bronopol was not carcinogenic in this study. The most frequently observed tumors were pituitary adenoma in both sexes and mammary fibroadenoma in the females, but the incidence (number of rats with tumor/number of rats examined) was dose-unrelated and was lowest in the high-dose group. The incidence of pituitary adenoma in the control, low-dose, mid-dose and high-dose male rats was 10/43 (23%), 14/43 (32%), 7/42 (17%) and 2/41 (5%), respectively. The corresponding incidences in the female rats were 20/44 (45%), 21/45 (47%), 23/42 (55%) and 14/38 (37%), respectively. The incidence of mammary fibroadenoma in the control, low-dose, mid-dose and high-dose female groups was 35/44 (79%), 36/45 (80%), 30/42 (71%) and 19/38 (50%), respectively. Therefore, the NOAEL for the systemic toxicity of bronopol in the present study was 7 mg/kg bw/day (reliability 2). ([redacted] *et al.*, 1976; US EPA RED, 1995)

A.6.6.3.2. Chronic toxicity and carcinogenicity study in rats 2

In the carcinogenicity study on a second species, bronopol (purity ≥ 99.7%) was administered dermally to CFLP mice of Swiss origin (52/sex/dose) on Monday, Wednesday and Friday of each week, for 80 weeks. Doses were 0.3 mL/mouse/day of 0, 0.2% and 0.5% solutions in acetone (90%):water (10%), corresponding to 0, 20 mg/kg bw/day and 50 mg/kg bw/day, which are considered sufficient. The following observations/examinations were included in the study: body weight, food and drinking water consumption, macroscopic investigations (skin lesions, cataracts and/or palpable masses), clinical signs, mortality, skin reactions, pathology, gross pathology at necropsy (all superficial tissues including urogenital orifices, tails, auricles, auditory meatus, mammary tracts and cervical subcutaneous structures, nares, mouth, tongue, pharynx and auditory region, brain, pituitary gland and the cranial nerves, regional lymph nodes, mammary glands and salivary glands, abdominal and thoracic contents, urinary bladder, thymus, the lymph nodes and the heart, mucosa of the esophagus, intestines and uterus, all pleural surfaces, liver, kidney, gonads, adrenals, thyroid, intra-abdominal lymph nodes and accessory reproductive organs) and histopathology (all abnormal tissues, aorta, brain, mid-colon, pituitary, thymus, salivary glands, stomach, jejunum, liver, kidneys, spleen, heart, lungs, gonads, uterus, adrenals,

seminal vesicles, skeletal muscle, skin (untreated), treated skin areas, tongue, urinary bladder, gall bladder, lymph nodes, mammary gland, sciatic nerve, thyroid, pancreas, bone, bone marrow, eye, blood smears). The only treatment-related effects observed were a minimal hair loss in the high-dose (0.5%) in some males and females periphery of the shaved area during the first 3 weeks of treatment, and a decreased body weight gain in the high-dose males especially during weeks 26-52 when they gained 47% less weight than controls. Bronopol was not carcinogenic in this study. The numbers of tumor-bearing male mice in the control, low-dose and high-dose groups were 24/50 (48%), 21/50 (42%) and 23/50 (46%), respectively, females were 25/51 (49%), 18/50 (36%) and 22/49 (45%), respectively. The most frequently observed tumors were lymphoma in the lymphoreticular system and lung tumors reported only as tumors Grades 1, 2, 3 or 4. The incidence of lymphoma in the control, low-dose and high-dose male mice was 5/50 (10%), 3/50 (6%) and 6/50 (12%), respectively, in females 5/51 (9.8%), 8/50 (16%) and 10/49 (20.4%), respectively. The incidence of lung tumors (all grades) in each one of the male groups was 13/50 (26%), in females 10/51 (19.6%, control), 9/50 (18%, middle) and 11/49 (22.4%, high dose group). None of the tumor incidences observed in the treated male and female mice was statistically significant when compared with the control mice. Non-neoplastic lesions were observed most frequently in the lungs (lymphoid aggregations and/or alveoli with macrophages) and liver (vacuolated, distended or degenerated hepatocytes) of male and female mice, and in the ovaries (cysts), but were treatment-unrelated. Mortality of treated males was slightly increased compared to control males (after 80 days: 50% mortality in the 0.2% test group and 48% mortality in the 0.5% group, versus 36% mortality in control). 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 could be evidenced. The gross pathological and histopathological examination of both, animals that died during the experiment and animals that were sacrificed at test ending, revealed no treatment-related abnormalities. The NOAEL for systemic toxicity was 0.2%. An increased incidence of skin papilloma was reported for the highest tested concentration of bronopol (0.5%); however, these tumors as non-neoplastic lesions 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 (reliability 2). ([redacted] *et al.*, 1975; US EPA RED, 1995)

A.6.6.4. Developmental toxicity studies

A.6.6.4.1. Prenatal developmental toxicity study in rabbits 1 (OECD TG 414)

Groups of 26 time-mated female New Zealand White rabbits were administered bronopol by gavage at targeted dose levels of 0, 3, 10, or 30 mg/kg bw/day on gestation days (GD) 7-27. In-life parameters evaluated for all groups included: clinical observations, body weight, body weight gain, and feed consumption. On GD 28, all surviving rabbits were euthanized and examined for gross pathologic alterations and changes in liver, kidney, and gravid uterine weight. The number of corpora lutea, uterine implantations, resorptions, and live/dead fetuses were determined. All fetuses were weighed, sexed, and examined for external and visceral alterations. Also, the heads were examined for craniofacial alterations by serial sectioning for approximately one half of the fetuses in each litter, while skeletal examinations were evaluated on all fetuses. Maternal and developmental effects were observed at 30 mg/kg bw/day. The maternal effects consisted of body weight loss at the start of treatment, sporadic occurrences of slightly reduced feed consumption, slightly decreased body weight gains over the entire treatment period (GD 7-28), decreased fecal output, and noisy respiration. The developmental effects at the 30 mg/kg bw/day dose level consisted of a marginal increase in the overall incidence of axial skeletal malformations (7 fetuses from 4 litters) relative to controls (2 fetuses from 2 litters). However, six of the seven fetuses with axial skeleton malformations came from three does which exhibited significant maternal toxicity during the period of axial skeleton pattern formation (*i.e.*, somitogenesis). Therefore, the marginal increase in axial skeletal malformations in the 30 mg/kg/day group was considered to be the result of maternal toxicity exacerbating the normal background incidence of these axial skeletal alterations. There were no treatment-

related maternal or developmental effects at the lower doses. The maternal NOEL and developmental NOEL were considered to be 10 mg/kg/day. ([redacted] and [redacted], 2007)

A.6.6.4.2. Prenatal developmental toxicity study in rabbits 2 (OECD TG 414)

Effects of bronopol (> 98%) on pregnant female rabbits and embryo-foetal development were assessed when administered at dose levels of 0, 5, 20, 40, and 80 mg/kg bw/day by oral gavage to mated female rabbits from day 7 through day 19 post coitum (p.c.). Vehicle control animals were dosed with the vehicle alone (acidified (pH 4) water). A post-exposure period of 9 days was included in the study (days 20-28). Body weight, food consumption, clinical signs and mortality was recorded during the study. Post mortem examination, including gross macroscopic examination of all internal organs, was performed including gravid uterine weight, number of corpora lutea, number and distribution of implantations (implantation sites, resorptions). Implantations were classified in early resorptions, late resorptions, dead and live fetuses. Conception rates as well as the pre- and post-implantation losses were calculated. No treatment-related mortalities were reported. Two moribund females (one of the 5 mg/kg bw/day group and one of the 80 mg/kg bw/day group) were sacrificed *in extremis*, but bronopol treatment was not considered to be the direct cause of morbidity. Regarding the animals sacrificed at scheduled time (day 28 of pregnancy), the dams of the 80 mg/kg bw/day group showed a reduction in size and quantity of fecal pellets throughout most of the treatment period; this effect was related to the decreased food consumption of these animals. No further treatment-related symptoms were seen. Maternal body weight gain in the high-dose group was decreased compared to control, mean food consumption was significantly lower than for control (day 7 to 11: -38%). Necropsy of the dams that were sacrificed at scheduled time revealed no treatment-related abnormalities; necropsy of the high-dosed female sacrificed *in extremis* revealed extensive ulceration of the gastric mucosa. As the deterioration of the health state of this female had commenced prior to starting bronopol treatment, bronopol was not considered to be the direct or primary cause for this state, but it cannot be ruled out that the treatment might have exacerbated the bad state of health of the animal and might have contributed to the development of the gastric ulceration. No signs of maternal toxicity were seen in the remaining test groups (5, 20 and 40 mg/kg bw/day). All females including those, which were sacrificed *in extremis* were pregnant; each group comprised between 17 and 19 litters. No adverse treatment-related effects on corpora lutea, implantations, number of live fetuses, and sex ratio were reported. Embryotoxicity could be evidenced in the 80 mg/kg bw/day group. In this group, the mean fetal weight (both sexes) was significantly decreased compared to control. The decrease in fetal weight was indicative of embryonic growth retardation, which again probably related to the decreased food consumption and body weight gain reported for the dams of this group. Gravid uterine weights showed no treatment-related effects. A mean incidence of 6.9% of fetuses showing major abnormalities was reported for the 80 mg/kg bw/day group (control: 0%; this conspicuous low incidence in control group was unusual and incidental); the difference, however, was not statistically significant. The major abnormalities were of great variability and belong to the most common fetal abnormalities occurring spontaneously in the rabbit strain used; however, the conspicuously elevated incidence reported for the 80 mg/kg bw/day group probably was due to treatment. Further treatment-related effects seen in the 80 mg/kg bw/day group included increased incidences of runted fetuses (related to the growth retardation observed in this group), fetuses with minor skeletal abnormalities, and of fetuses with non-ossification of the fore- and hind limb epiphyses; these abnormalities were indicative of a general retardation of the fetal skeletal ossification and growth. Embryotoxicity could not be evidenced in the 5, 20 and 40 mg/kg bw groups. The NOAEL for maternal and embryotoxic effects was 40 mg/kg bw/d, thus, no embryotoxicity was observed in the absence of marked maternal toxicity (reliability 1). ([redacted], 1991a; US EPA RED, 1995)

A.6.6.4.3. Prenatal developmental toxicity study in rabbits 3 (OECD TG 414)

In advance to the above-mentioned study, a range-finding study was conducted under

similar conditions. Dosing of bronopol (>99.8%) was done accordingly in dose levels of 0, 1, 3, 10, 20, 40, 80 and 160 mg/kg bw/day by oral gavage (group 7 received 80 mg/kg bw/d on days 7 to 13 of pregnancy and 160 mg/kg bw/d on days 14 to 16 of pregnancy). Vehicle control animals were dosed with the vehicle alone (acidified (pH4) water). Study parameters were closely related to the above-mentioned main study. The highest test dose of 160 mg/kg bw which was given to the females of group 7 starting from day 14 of pregnancy, was found to be too high as this dosage resulted in mortality and body weight loss. No treatment-mortalities were reported for the remaining dosages including 80 mg/kg bw/day (group 7, day 7 to 13 of pregnancy). One moribund female treated with 80 mg/kg bw was sacrificed *in extremis* on day 14 of pregnancy; the bad state of health of this animal already was seen prior dosing and therefore was not treatment-related. Signs indicative of maternal toxicity were seen in females treated with 80 mg/kg bw/day of bronopol, and mainly consisted of loss in body weight gain, decrease in food consumption, and development of hemorrhages and ulceration in the gastric mucosa, as revealed by necropsy. The females of the groups treated with up to 40 mg/kg bw/day of bronopol showed no treatment-related adverse effects. No adverse treatment-related effects on pregnancy, corpora lutea, implantations, number of live fetuses, and sex ratio were reported. No fetal data were reported for group 7 as the dams died or were sacrificed prior to day 28 of pregnancy. In the groups treated with up to 40 mg/kg bw/day of bronopol, all considered data (embryonic growth, mean fetal weight, sex ratio, major and minor abnormalities) were not affected by the treatment with bronopol. The NOAEL for maternal effects and NOEL for embryotoxic effects was 40 mg/kg bw/d, thus, 80 mg/kg bw/d was used as high-dose in the main study above (reliability 2). ([redacted], 1991b; US EPA RED, 1995)

A.6.6.4.4. Prenatal developmental toxicity study in rats 1 (OECD TG 414)

Effects of bronopol (> 99.5%) on pregnant female CD rats and embryo-fetal development were assessed in an additional range finding study. The study consisted of two phases including different dose levels. Phase I was performed with 4 dose levels of 0, 3, 10, 30 and 100 mg/kg bw/d and Phase II with additional three dose levels of 60, 80 and 100 mg/kg bw/d. Test substance was administered by oral gavage to mated female rats from day 7 through day 15 post coitum (p.c.). Vehicle control animals were dosed with the vehicle alone (acidified (pH4) water). Body weight, food consumption, clinical signs and mortality were monitored during the study. Post mortem examination, including gross macroscopic examination of all internal organs, was performed including gravid uterine weight, number of corpora lutea, number and distribution of implantations (implantation sites, resorptions). Implantations were classified in early resorptions, late resorptions, dead and live fetuses. Conception rates as well as the pre- and post-implantation losses were calculated. The live fetuses were weighed and were examined for sex. All fetuses were examined for external abnormalities and were then fixed in neutral buffered formaldehyde (10%). The mean fetal bodyweight and the sex ratio were calculated for each litter. Signs indicative of maternal toxicity were observed from 30 mg/kg bw, up to the highest test dose of 100 mg/kg bw/d of bronopol. In fact, these signs mainly consisted of a reduction in body weight gain, a reduction in food consumption, a poor state of health (100 mg/kg bw/d) and impaired respiration (100 mg/kg bw/d). No signs of toxicity were seen at the lowest tested doses of 3 and 10 mg/kg bw/d, respectively. Three females of the 100 mg/kg bw groups were sacrificed *in extremis*. Two of these females were sacrificed during phase I of the study, necropsy revealed red lung lobes in both cases and in one female hemorrhaging of the stomach glandular mucosa and gas in the caecum also were reported. The third 100 mg/kg bw female which was sacrificed *in extremis* during phase II of the study had an extensive ulceration within the glandular stomach mucosa and colon contents were dehydrated; this female was not gravid. No signs of developmental toxicity could be evidenced; in fact, all considered parameters were inconspicuous. The NOAEL for maternal toxicity was 10 mg/kg bw/d and the NOEL for teratogenic effects was 100 mg/kg bw/d (reliability 2). ([redacted], 1993)

A.6.6.4.5. Prenatal developmental toxicity study in rats 2 (OECD TG 414)

Based on the results of the above-mentioned range-finding study the dosing in the main study (GLP-conform) was 0, 10, 28, and 80 mg/k bw/d from day 6 to day 15 of pregnancy. Bronopol (> 95%) was administered by gavage to CD rats. The study design was similar, fetal examination included external and visceral examinations according to EPA OPP 83-3 guideline. At the highest tested dose of 80 mg/kg bw/day a significant but transient decrease in body weight gain was reported for days 6 to 7 of pregnancy (1 ± 5 g versus 5 ± 3 g for control). Thereafter, body weight gain in this group turned back to control level. No further treatment-related effects were seen. None of the considered pregnancy data was affected by the treatment. No adverse effect of bronopol treatment on embryonic and fetal development could be evidenced. In fact, advanced ossification of the sacral neural arches in the 80 mg/kg bw group, and advanced ossification of the forelimb phalanges in both the 28 and the 80 mg/kg bw groups were reported; these findings might have been related to the treatment, but as they still were within background mean range, they were not seen as conspicuous. There was no evidence of developmental toxicity at any of the dose levels tested. The NOAEL for maternal toxic effects was 80 mg/kg bw/d and the NOEL for teratogenic effects was 80 mg/kg bw/d (reliability 1). ([redacted], 1995; US EPA RED, 1995)

A.6.6.4.6. Peri- and postnatal developmental toxicity study in rats

In a non-guideline study the effect of bronopol (>98%) on peri- and postnatal development was investigated. Bronopol was administered by gavage to pregnant CD rats in dose levels of 0, 10, 20, and 40 mg/kg bw/d in the preliminary test study and of 0, 20 and 40 mg/kg bw/d in the main study. Dosing was started on day 15 of pregnancy and was continued until day 12 post parturition. Examinations included clinical signs and mortality, body weight, pregnancy (pregnancy rate, pregnancy duration and dystocia during parturition), litter (dead and/or abnormal pups) and pup data (body weight again on d4, d12 and d21 post-partum; sacrificed on d21; examination for external and internal abnormalities as well as for the sex (gonadal inspection)), necropsy of the dams. In the preliminary test, no treatment-related mortality was observed, and no treatment-related clinical symptoms were reported. In fact, one dam of the 40 mg/kg bw group was sacrificed *in extremis* on day 24 because of dystocia; necropsy revealed one dead pup in the vagina whereas further 15 dead pups were found in the uterus which was filled with blood. No treatment-related effects were reported for body weight, pregnancy rate and pregnancy duration. Except for the dam of the 40 mg/kg bw group which was sacrificed *in extremis*, no litter losses were reported. Litter size, pup mortality, litter weights and mean pup weights showed no treatment related effects; pups were free from abnormalities. In the main study, two cases of mortality were reported, which respectively occurred in the 20 and the 40 mg/kg bw groups. In both cases, the dams died during the postpartum period; both dams had low initial body weights when compared to the remaining animals. The mortalities were not considered to be treatment-related. No treatment-related clinical symptoms were reported. Body weight, pregnancy rate and pregnancy duration were unaffected by the treatment. Regarding litter and pup data, one dam of the control group, two dams of the 20 mg/kg bw group and one dam of the 40 mg/kg bw group showed total litter loss. The litter losses were not treatment-related. Compared to control, pup mortality was slightly increased in the treated groups from day 4; in fact, the differences were statistically significant on day 12 and 21 for the 20 mg/kg bw group, and on day 21 for the 40 mg/kg bw group. These findings however were not considered to be of toxicological relevance as the pup mortality in the control group was unusually low when compared to laboratory standard range; furthermore, total litter losses were similar in the control and the high dose group, so no dose-response relationship was observed. Litter and mean pup weights in the treated groups were slightly below control values from day 12 post-partum and were below the laboratory standard range on day 21; however, the differences were of no statistical significance. Neither the dams nor the pups showed treatment-related effects at the dose levels tested, the maternal and developmental NOAELs were 40 mg/kg bw/d (reliability 2). ([redacted], 1973a)

A.6.6.4.7. One-generation reproduction toxicity study (OECD TG 415)

In a one-generation study in rats, bronopol (>98%) was administered by gavage to CD rats in doses of 0, 20, or 40 mg/kg bw/d for 19 weeks. Males were exposed before mating for 63 days and females for 14 days. Distilled water was used as vehicle, the control group received vehicle alone. Examinations included clinical signs and mortality, body weight, mating performance, pregnancy rate, and gestation period. A part of the pregnant females was sacrificed on GD13, number of corpora lutea, pre- and postimplantation losses, number of viable offspring were determined. From pregnant females that could litter normally, the pups were counted, weighed and examined for abnormalities. Five cases of death were reported for the parental animals (one male and one female in the 20 mg/kg bw group; one male and two females in the 40 mg/kg bw group), which however, could not be attributed to the treatment. No treatment-related symptoms of toxicity were seen. Body weight changes of all treated females (pre-mating, gestation, lactation and post-lactation period) and of the males treated with 20 mg/kg bronopol, were within control range and therefore inconspicuous. For the males of the 40 mg/kg bw group and starting from week 2 of treatment, weight gain was found to be slightly but clearly below control values. All considered reproduction parameters were within control range and showed no treatment-related effects; no treatment-related abnormalities were seen. The fertility and reproductive performance of the rats as well as the pre-and post-natal development of the young were not adversely affected by the repeated oral treatment with bronopol at doses up to 40 mg/kg bw/d (NOEL) (reliability 2). ([redacted], 1973b).

A.6.6.4.8. Fish early life stage(ELS) toxicity test (OECD TG 210)

To determine the chronic toxicity of Bronopol to the early life stages (ELS) of rainbow trout (*Oncorhynchus mykiss*), a GLP study was conducted according to OECD TG 210 (version adopted 17 Jul 1992) [[redacted] 1996].

The currently available assay (A7.4.3.2_01, [redacted], 1996) has a shorter duration (49 days) that the recommended in OECD TG 210 (ELS) (60 days post-hatch). Information not available about the life stage of the fish during the assay.

Although this assay has not endocrine specific endpoints. There are limited evidences to suggest that is responsive to certain thyroid-disrupting chemicals.

Adverse effects on survival, growth and development were evaluated and recorded. 40 fish eggs per replicate were exposed in duplicates to bronopol concentrations ranging from 2.25 mg/L to 40 mg/L (nominal) under flow-through conditions (49 to 70 mL per minute) for 49 days. The nominal concentrations of 2.3, 4.4, 6.9, 11.5 and 40.0 mg/L correspond to mean measured concentrations of 0.44, 0.07, 0.29, 1.18, 1.94 and 7.48 mg/L. A concentrated stock solution of 0.562 g/L bronopol in reverse osmosis deionised water was prepared each day and passed into the proportional dilution apparatus (ELL SOP 297) where it was mixed with dilution water to achieve the test concentrations. The control consisted of test medium without bronopol. No analytical monitoring performed on actual concentrations.

Mortality rates and any sub-lethal effects such as deformities were recorded daily. Low rates of deformities among the hatched larvae and fry were observed including deformed spines leading to a bent or spiral condition. The number of deformed fish per replicate (incl. control) did not exceed two; thus, the deformities were considered to be not treatment-related. Based on the experience from previous experiments, a low level of deformities is common for trout fry. The swim bladder inflation was not reported in detail, but only swim-up was noted.

At the end of the test, all surviving fish were weighed and measured, and the individual condition indices were calculated. These data were statistically analysed to determine whether any of the exposure groups differed significantly ($p=0.05$) from the control. Bronopol showed no sublethal adverse effects regarding development (length, weight, and condition index) in all tested concentrations up to (and incl.) the NOEC, which is based on mortality. No thyroid related effects have been observed. However, 0% immobility was observed after 24 hours and 4% immobility was determined after 48 hours. Survival of

early-life-stage rainbow trout was significantly affected by the chronic treatment with bronopol under flow through conditions at a nominal test concentration of 40 mg/l. Mortality for all other test concentrations (2.25 to 21.50 mg/l) was within the control range. At highest nominal test concentration, mortality reached 57.5%.

According to scenario N of Table C.2.8. "Fish, Early-Life Stage (FELS) Toxicity Test (OECD TG 210): Guidance for scenarios of combinations of results with existing data" and §402 in OECD TG 150 it can be concluded that due to the historical and recent data bronopol is not disrupting the thyroid system, although the test does not provide any specifically information to EDCs alone, because this study does not include biomarkers for endocrine activity, but it could be relevant to adversity of the ED assessment.

Due to several deviation from the OECD guidance and the relevance of the assessment, the obtained results are considered supportive information. Besides, the results should be considered carefully due to the low recoveries rate.

A.6.6.4.9. Fish, Juvenile Growth Test (OECD TG 215)

The chronic toxicity of the test item Bronopol to rainbow trout (*Oncorhynchus mykiss*) was investigated in a 28-day flow-through test according to OECD TG 215 (version adopted 21 January 2000) conducted under GLP conditions [redacted 2007]. Juvenile rainbow trout were exposed to aqueous test media containing the test item at various concentrations under defined conditions. A blank control containing only test medium but no test item was tested in parallel. From the nominal concentrations of 0.32, 1.0, 3.2, 10 and 32 mg/L only the three highest concentration (3.2 to 32 mg/L) were analysed resulting in mean measured concentrations of 2.6 (82% of nominal), 9.3 (93% of nominal) and 30 mg/L (94% of nominal). The concentrations of the test item in the test media were maintained by dosing concentrated aqueous test item solutions (application solutions) into test water (14 mL per hour spread over 20 single dosages) by means of automatic dispenser units (HAMILTON digital dispenser). The application solutions were prepared one day prior to test start and renewed every 6 to 8 days (at Day -1, 7, 13 and 21). The application solution used for the highest test concentration was prepared by completely dissolving nominal 109.7 g of the test item in 4000 mL of purified water using intensive stirring for 10 minutes. In a series of subsequent dilution steps, this application solution was diluted with purified water to prepare the application solutions used for the lower test concentrations. For stabilisation of the application solutions during their use, few drops of chlorinated acid were added to reduce the pH value to 4. The stability of the application solutions was confirmed by recovery rates of 94 to 105%.

The fish were fed with a high amount of 4% food relative to their mean body wet weight to obtain exponential fish growth. The body wet weight of each fish was measured at the start and at the end of the test to determinate the fish growth rates during the exposure period. Additionally, the mortality and visible abnormalities were recorded daily.

A statistical evaluation of the mean body wet weight and the mean pseudo specific fish growth rate for body wet weight was not performed. The NOEC and LOEC were determined directly from the raw data. Bronopol showed no sublethal adverse effects regarding development (body wet weight, and growth rate) in all tested concentrations up to (and incl.) the NOEC, which is based on mortality. At the measured test concentration of 9.3 mg/L one fish died at Day 16. It was therefore set as LOEC. In the highest test concentration 50% of the fish died during the test. The fish in the blank control exhibited a healthy development with an increase in mean wet weight by a factor of 3.1. No ED related effects have been observed in any of the treatments.

A.6.6.4.10. *Daphnia magna* reproduction test (OECD TG 211)

In the available Daphnia reproduction test according to OECD TG 202 (1984), no response of endocrine disrupter to apical endpoints were observed or determined. No sublethal adverse effects with respect to presence of eggs in the brood pouch of parental daphnia, number and conditions of newborn, unhatched eggs and incident of immobility in the F1

generation were observed. The NOEC is based on immobility. It is a GLP compliant guideline study without deficiencies [[redacted] 1992].

In the available Daphnia reproduction test according to OECD TG 211 (1998), no mortality due to the exposure of Bronopol was observed up to the highest treatment. The parental daphnids in the highest treatment showed a reduction in body length compared to the mean body length of the parental daphnids in the pooled control (blank and solvent control) and produced less offspring than the daphnids in the controls. No sublethal adverse effects with respect to presence of eggs in the brood pouch of parental daphnia, unhatched eggs and incident of immobility in the F1 generation were observed. The NOEC is based on immobility. It is a GLP compliant guideline study without deficiencies [[redacted] 2004].

The primary purpose of the OECD TG 211 guideline is to assess the number of offspring. The endpoint of induction of male offspring is (still) optional (with reference to the current version of 2012). An additional criterion for the validity of the test is less than 5 % male offspring in the control treatment.

No sex differentiation of the offspring was not performed in this study.

Four replicates with 10 daphnids each resulted in a total of 40 daphnids per test concentration. No possibility to link the offspring from a replicate to the respective parental adult daphnids. The study reports there was no information provided that any size differences occurred in the parental daphnids at the end of the exposure period. However, the absence of any reported visual size differences among the adult daphnids after 21 days of exposure supports the conclusion that the test substance had no significant impact on the sex ratio of the daphnids.

This is also supported by the fact, that no ephippia were reported during the test period. Due to the absence of ephippia, it can be concluded that the female daphnids were neither stressed nor were any male daphnid present at any time during the 21d exposure period. Despite that assay is not included in the ED assessment strategy described in OED GD 150, that assay could be considered just as supportive information.

A.6.6.4.11. Other test data

In a non-guideline study the effect of Bronopol (>98%) on peri- and postnatal development was investigated. Bronopol was administered by gavage to pregnant CD rats in dose levels of 0, 10, 20, and 40 mg/kg bw/d in the preliminary test study and of 0, 20 and 40 mg/kg bw/d in the main study. Dosing was started on day 15 of pregnancy and was continued until day 12 post parturition. Examinations included clinical signs and mortality, body weight, pregnancy (pregnancy rate, pregnancy duration and dystocia during parturition), litter (dead and/or abnormal pups) and pup data (body weight again on d4, d12 and d21 post-partum; sacrificed on d21; examination for external and internal abnormalities as well as for the sex (gonadal inspection)), necropsy of the dams. In the preliminary test, no treatment-related mortality was observed, and no treatment-related clinical symptoms were reported. In fact, one dam of the 40 mg/kg bw/d group was sacrificed in extremis on day 24 because of dystocia; necropsy revealed one dead pup in the vagina whereas further 15 dead pups were found in the uterus which was filled with blood. No treatment-related effects were reported for body weight, pregnancy rate and pregnancy duration. Except for the dam of the 40 mg/kg bw/d group which was sacrificed in extremis, no litter losses were reported. Litter size, pup mortality, litter weights and mean pup weights showed no treatment-related effects; pups were free from abnormalities. In the main study, two cases of mortality were reported, which respectively occurred in the 20 and the 40 mg/kg bw/d groups. In both cases, the dams died during the post-partum period; both dams had low initial body weights when compared to the remaining animals. The mortalities were not considered to be treatment-related. No treatment-related clinical symptoms were reported. Body weight, pregnancy rate and pregnancy duration were unaffected by the treatment. Regarding litter and pup data, one dam of the control group, two dams of the 20 mg/kg bw/d group and one dam of the 40 mg/kg bw/d group showed total litter loss. The litter losses were not treatment-related. Compared to control, pup mortality was slightly increased in the treated groups from day 4; in fact, the differences were statistically significant on day 12 and 21

for the 20 mg/kg bw/d group, and on day 21 for the 40 mg/kg bw/d group. These findings however were not considered to be of toxicological relevance as the pup mortality in the control group was unusually low when compared to laboratory standard range; furthermore, total litter losses were similar in the control and the high dose group, so no dose-response relationship was observed. Litter and mean pup weights in the treated groups were slightly below control values from day 12 post-partum and were below the laboratory standard range on day 21; however, the differences were of no statistical significance. Neither the dams nor the pups showed treatment-related effects at the dose levels tested, the maternal and developmental NOAELs were 40 mg/kg bw/d (reliability 2) [1973a].

In two other studies Bronopol was tested in a drinking water in an equimolar (10 µM each) mixture together with 22 other chemicals (1,2,3-trimethylbenzene, 2-(2-methoxyethoxy)ethanol, 2-ethylhexanol, 2-methyl-4-isothiazolin-3-one, acrylamide, benzene, cumene, diethanol-amine, ethoxylated nonylphenol, ethoxylated octylphenol, ethylbenzene, ethylene glycol, ethylene glycol monobutylether, naphthalene, N,N-dimthylformamide, phenol, propylene glycol, sodium tetraborate decahydrate, styrene, toluene, triethylene glycol, xylenes). Ten-week-old C57/BL6 mice were time mated and denoted as gestational day (GD) 0 on the day of vaginal plug. On GD 11, dams were provided with experimental treatments in their drinking water. Test concentrations included a 0.2% ethanol vehicle, 166.67 µg/mL flutamide control (androgen antagonist; estimated exposure 50 mg/kg/ d), and four concentrations of a mixture of 23 oil and gas operation chemicals, with each individual chemical present at 0.003, 0.03, 0.3, 3 mg/kg bw/d. Experimental doses were provided until birth and dams were then reverted to standard acidified water. Due to the fact that Bronopol was tested only in a mixture together with 22 other (toxicologically potent) chemicals, no conclusions can be drawn from those experimental set-ups regarding Bronopol-specific effects [*et al.* 2015; *et al.* 2016]. This is also true for an *in vitro* study of the same group of authors investigating adipogenesis mechanisms in 3T3-L1 fibroblasts [*et al.* 2018].

Across the available repeated dose toxicity studies, a number of organ weight and histopathological examinations were performed. Organ weight measurements in these studies included adrenal, brain, ovary, pituitary, prostate, seminal vesicle, testis, thyroid/parathyroid, and/or uterus. While changes in organ weights were seen, these were generally secondary to decreases in body weight and were without a consistent pattern across available studies. Histopathological examinations in repeated dose toxicity studies included adrenal, cervix, epididymis, mammary glands, ovary, pituitary, prostate, seminal vesicles, testis, thyroid, uterus, and/or vagina. No treatment-related changes were observed in any organ in any study, demonstrating an absence of effect of Bronopol on these organs, even at doses in excess of the maternal/parental toxic dose and/or for durations of up to 2 years.

The endocrine disruptive potential of Bronopol has recently been assessed in *Xenopus laevis* [*et al.* 2018]. The tadpole developmental stage is 51-52 and were exposed during 3 weeks. Then the 6-week-old tadpoles are still at premetamorphic stages (stage 55–56). More advanced tadpoles that reached stage 56 were discarded to minimize effect of metamorphosis on gene expression. Hence, the exposure took place during the NF 51-55. The results are referred to the premetamorphic tadpoles NF 54-55. The tadpoles were treated with Bronopol as part of equimass mixtures (0.1, 1, 5 or 10 µg/L) of a total of 23 chemicals to simulate realistic environmental conditions. Water and chemicals were changed once a week. These 23 chemicals were selected based on their suspected endocrine activity towards any relevant hormone receptors analysed by *et al.* 2015 (listed in Table 1). In that paper, does not consider that Bronopol has Thyroid activity.

et al. 2018 stated "To reduce the complexity of the UOG mixture, we selected 6 of the 23 chemicals based on their putative thyroid related activity in tadpole developmental stage. Thyroid hormone signalling is critical for metamorphosis and development, and thyroid-disrupting compounds have been reported to affect frog development (and , 2012). Four chemicals (ethoxylated, nonylphenol, ethoxylated octylphenol, ethylene glycol, and napthalene) exhibit wide EDC activity including thyroid receptor antagonism (*et al.*, 2015, 2016c), cumene can induce thyroid tumor (2009), and sodium

tetraborate decahydrate is implicated in birth defects ([redacted] *et al.*, 1998)". Bronopol was not included in the reduced list of 6 tested substances based on its unlikely T-modality effects.

It should be noted that although the conditions in this study can be considered environmentally relevant and represent a worst-case approach, it should be considered not relevant for the possible T-modality due to the absence of measurements of Bronopol (it is not considered to have T-effects based on [redacted] publication), since the results obtained are related to a mixture of 23 chemicals (including Bronopol). Hence, [redacted] *et al.*, 2015, 2016c, can be considered as supporting information to the unlikely T-modality effects from Bronopol.

A.6.7. LEVEL 5

Level 5 data comprises *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism.

A.6.7.1. Two-generation reproduction toxicity studies (OECD TG 416)

A.6.7.1.1. Two-generation reproduction toxicity study in rats 1

Groups of 27 male and 27 female Crl:CD(SD) rats were administered 0, 0.01, 0.05, and 0.15% bronopol in pH 4 acidified drinking water for approximately ten weeks prior to breeding, and continuing through breeding, gestation and lactation for two generations. These administered doses of bronopol corresponded to targets of 0, 10, 50, and 150 mg/kg/day. In-life parameters included clinical observations, feed consumption, body weights, estrous cyclicity, reproductive performance, pup survival, pup body weights, and puberty onset. In addition, post-mortem evaluations included gross pathology, histopathology, organ weights, oocyte quantitation and sperm count, motility and morphology in adults, and gross pathology and organ weights in weanlings.

Administration of 0.15% bronopol resulted in decreased water consumption (attributed to decreased palatability) throughout most of the study, along with secondary, minor decreases in feed consumption. Decreases in body weights occurred sporadically in both sexes and generations, although there was a consistent decrease in body weight gain in F0 and F1 females in the week prior to delivery. Microscopic examination of tissues from adult animals revealed minor changes in the kidneys, thyroid, stomach, and liver. Kidney effects were found in males and females of both generations and consisted of very slight-slight nephropathy corresponding with increased absolute and relative kidney weights. There was an increased incidence of very slight follicle dilatation of the thyroid correlating with increased absolute and relative thyroid weight for F0 females. Thyroids of F0 males and F1 males and females were not examined histopathologically due to an absence of change in thyroid weight in these animals. Other microscopic effects were of minor severity and restricted to F0 and F1 females. These changes included erosions, ulcer and/or inflammation of the glandular stomach, and vacuolization of scattered individual hepatocytes (fatty change) with no corresponding liver weight changes in high dose F0 females.

Similar to the high dose group, administration of 0.05% bronopol resulted in statistically identified decreased water consumption (attributed to decreased palatability) in F0 males and females during the first half of the study (days 1-57 and 1-50, respectively). Water consumption for F1 parental animals was not decreased in the 0.05% dose group. Histopathologic effects were limited to an increased incidence of very slight- slight nephropathy restricted to F1 parental males, but no change in organ weight. Also, histopathologic examination of the thyroid in F0 females revealed a marginal increase in the incidence of very slight follicular dilatation, without corresponding thyroid weight change. There were no other systemic or histopathologic treatment related effects in the mid-dose group. There were no treatment related systemic or histopathologic effects in the low-dose animals administered 0.01% bronopol.

Reproductive effects occurred only at the 0.15% dose level and were limited to two cases of difficult delivery (dystocia) in the F0 dams, and increased postimplantation loss (18.8

versus 6.8% in controls) and associated decreases in gestation survival and stillborn pups, along with two total litter losses in the F1 dams. These reproductive effects were attributable to six 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.

In conclusion, the systemic NOEL was 0.01% (10 mg/kg/day) bronopol and the NOEL for reproductive toxicity was 0.05% (50 mg/kg/day) bronopol. ([redacted] *et al.*, 2008).

A.6.7.1.2. Two-generation reproduction toxicity study in rats 2

In a range-finding study (following the same guidance as the definitive study (according to the SOP of [redacted])) that was conducted in advance to a two-generation study with bronopol, bronopol (99.9%) was administered to male and female CD rats (5/sex/group) via the drinking water at doses of 0, 0.025%, 0.05%, 0.1%, and 0.2% corresponding to 0, 17.2, 31.8, 64.3 and 113.3 mg/kg bw/day in males and 0, 30.9, 52.7, 95.7, and 193.7 mg/kg bw/d in females. Mating (10 d) was conducted after 14 days of treatment. Duration of exposure was 5-6 weeks in total. Examinations throughout the study included clinical signs and mortality, body weight, food and water consumption, mating performance, fertility indices for both, males and females, and gestation length, litter size, number of stillbirths, number of live births and gross abnormalities. The pups were checked twice daily for mortality and clinical symptoms of toxicity. They further were examined for gross abnormalities at birth and on day 3 of lactation. Body weights of the pups were recorded on day 3 of lactation (*i.e.* prior to sacrifice). On day 3 of lactation, all parental animals and pups were sacrificed for the purpose of necropsy. Of the parental animals, one moribund male of the 200 mg/kg bw/day group was sacrificed *in extremis* on week 2 of treatment; necropsy revealed ulcers and thickened mucosa in the non-glandular stomach, as well as bloody gastrointestinal contents. No further mortalities were reported.

Except for the moribund animal mentioned above none of the treated animals showed treatment-related clinical signs of toxicity. Body weight gain for the treated males was below control values, though some individuals from all treated groups showed weight changes similar to control animals. Mean food consumption of the males of the 200 mg/kg be group was reduced during the first week of treatment; thereafter, food consumption in this group turned back to control range. Water consumption for the males was reduced in all treated groups throughout the whole treatment period. In all groups including control, some females lost or gained little weight during the first two weeks of the experiment. Mean food consumption of the females of the 200 mg/kg be group was reduced during the first week of treatment. Water consumption for all treated females was reduced during the first week of treatment but turned back to control level on week two. Evaluation of weight change, food consumption and water consumption in females during the gestation phase was considered not to be appropriate because the animals became pregnant at different times and therefore, they were not all at the same stage of pregnancy at the time points when the parameters were measured. Necropsy of the sacrificed animals was inconspicuous except for one female of the 100 mg/kg bw/day group that displayed a cavity in the liver.

Due to the reduced water consumption, the test substance intake of the treated males was below the expected dosages and was as follows: 17.2, 31.8, 64.3 and 113.3 mg/kg bw/day; for the females, the mean achieved dosages were 30.9, 52.7, 95.7 and 193.7 mg/kg bw/day.

Copulatory interval, fertility indices and duration of gestation were not affected by the treatment. An increased post implantation loss was reported for the 50 and the 200 mg/kg bw/day groups; however, as no such decrease was observed for the intermediate group (100 mg/kg bw/day) and no dose-effect relationship was evident, this effect was not considered to be treatment-related. None of the considered litter parameters showed treatment-related effects and the pups showed no treatment-related abnormalities. On the basis of these results, for the main two-generation reproduction study with rats, the nominal doses selected were 25, 70 and 200 mg/kg bw/day (reliability 1). ([redacted], 1986)

A.6.7.1.3. Two-generation reproduction toxicity study in rats 3

In the main GLP-conform two-generation study, bronopol (>99.9%) was administered to male and female CD rats (13 males and 26 females per group) via the drinking water at doses of 0, 0.025%, 0.07%, and 0.2% corresponding to nominal doses of 0, 25, 70, and 200 mg/kg bw/d. The treatment was started 80 days prior mating, and mating duration was about 15 days. Treatment was continued thereafter until the end of the weaning period of the F1 young, which corresponded to the time point at which the F0 parents were sacrifice for necropsy. Treatment of the parental F1 rats was started at weaning and was continued until weaning of the F2 young and thereafter for 33 to 47 days.

Examinations throughout the study included clinical signs and mortality, body weight, food and water consumption, mating performance, fertility indices for both, males and females, and gestation length, litter size, number of stillbirths, number of live births and gross abnormalities, pathology. 10 selected parental F0 and F1 rats/sex/group as well as 5 selected F1b and F2b pups/sex/group were subjected to a complete external examination. Organs and tissues were examined in detail (adrenal, aorta, brain, colon, duodenum, epididymis, oesophagus, eye, heart, ileum, jejunum, kidney, liver, lung, lymph node, mammary gland, ovary, pancreas, pituitary, peripheral nerve, prostate, salivary gland, seminal vesicle, skeletal muscle, skin, spleen, stomach, testis, thymus, thyroid and parathyroid, trachea, urinary bladder, uterus and cervix, all gross lesions), organ weights were determined (adrenals, heart, kidney, liver, ovary, testis, thyroid/parathyroid) and a series of organs/tissues were further prepared for histopathological examination (adrenals, colon, epididymis, oesophagus, heart, ileum, jejunum, kidney, liver, lung, ovary, prostate, spleen, stomach, testis, thyroid, trachea, urinary bladder, uterus and cervix, and all gross lesions).

The achieved mean intakes of bronopol for the F0 and the F1 males and females were 22.5, 55.2 and 147 mg/kg bw/day. The lower achieved dosages were due to the reduced water consumption, which was observed in all treated groups. No treatment-related mortalities and symptoms were reported. Treatment-related reduced body weight and body weight gain were reported for high dose animals (F0 males & females, F0 females during gestation & lactation, F1 males & females, and F1 females during gestation). Treatment-related decreased food consumption was reported for all treated F0 males, for the high dose F0 females and high dose F0 females during lactation. Treatment-related decreased food consumption was reported for the high dose F1 males & females and for high dose F1 females during gestation & lactation. A dose-dependent, treatment-related decreased water consumption was observed for all treated F0 males & females and all treated F0 females during gestation; during lactation, decreased water consumption was seen in the mid & high dose groups but not in the low dose group. All treated F1 males & females as well as the F1 females during gestation (F2a mating) had decreased water consumption. During F2b gestation, only the mid and high dose females had decreased water consumption. During the F2a and the F2b lactation periods, water consumption of the F1 females was decreased in the mid and high dose groups but not in the low dose. Main findings were an increased incidence of progressive nephropathy, which was reported 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 substance as such. In high dose F1 parents, changes in liver and body weight were reported as apparently 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. For the F0 generation, the fertility index of the high dose females was slightly reduced compared to control, especially for F1a mating; all other reproduction and delivery parameters were inconspicuous. For the F1 generation, all reproduction and delivery parameters were inconspicuous. Survival indices of all F1a pups, of the mid and high dose F1b pups, and of all F2a and F2b pups were inconspicuous; survival index of the high dose F1b pups was slightly decreased. The high dose F1a, F1b, F2a and F2b pups showed a treatment-related significant decrease in mean body weights during lactation; the mean body weight of the mid dose F1b pups was slightly decreased towards the end of the lactation period. Mean body weights of the remaining pups were inconspicuous. Necropsy

of dead F1a, F2a and F2b pups revealed no treatment-related abnormalities. For F1b, necropsy of dead pups revealed pale hepatic lobes in two female pups of the same litter in the mid-dose group; one of these females further showed undeveloped renal papilla whereas the second one had distended ureters. In the high dose group, 3 dead pups were small in size. Necropsy of sacrificed F1a, F1b, pups revealed no treatment-related abnormalities. In fact, at necropsy, 22 high dose F1b pups were of decreased size; this however was related to the decreased body weights. Necropsy of F2a pups that were sacrificed at the end of revealed one case of abnormal eye in the mid dose group, sparse haircoat in ten pups of the same litter in the 25 mg/kg bw group and missing distal tail in one pup of respectively the mid and the high dose group. At necropsy of F2b pups, 1 control pup, 2 low dose pups and 14 high dose pups were small in size; 20 low dose pups (2 litters, 10 pups/litter) had sparse haircoat and 1 low dose pup was discolored purple; 1 high dose pup had a mass with hair loss on the top of its head. Within the present study, treatment-related effects referring to systemic toxicity were seen at all tested doses of bronopol but were particularly pronounced at the highest dose tested. Regarding reproductive or litter parameters, no significant treatment-related effects were reported; in fact, the effects reported at the highest test dose of 200 mg/kg bw rather resulted from the high systemic toxicity observed at this dose level than indicating reproductive toxicity.

The NOAELs (F0 and F1 generation) are 25 mg/kg bw/d for systemic toxicity and 70 mg/kg bw/d for reproduction toxicity, the NOAEL for the offspring is 200 mg/kg bw/d (reliability 1). ([redacted], 1987).

A.6.8. LINES OF EVIDENCE

The ED criteria for biocides are summarized in Annex I. The following discussion focuses on a weight of evidence based argumentation whether the ED criteria shown in Annex I are met for bronopol. This includes the initial analysis of the evidence as well as the analysis of the lines of evidence regarding thyroid disruption.

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12

A.6.8.1. Assessment of the integrated lines of evidence and weight of evidecen for potential estrogen / androgen-mediated or steroidogenesis-realted adversity and activity of Bronopol

The lines of evidence for Bronopol have been assessed using the Excel template according to Appendix E of the guidance (ECHA/EFSA, 2018). The lines of evidence for potential estrogen-/androgen-mediated or steroidogenesis-related activity are assembled, integrated and assessed as shown in Table 94 below.

In vitro mechanistic studies were available for Bronopol regarding endocrine activity. Furthermore, the endocrine activity was investigated using in-silico methods. None of the models and mechanistic studies predicted or showed, respectively, a binding of Bronopol to or activity of Bronopol on ER, AR. Furthermore, Bronopol had no effect on steroidogenesis, i.e showed no inhibition on aromatase and thyroperoxidase. Thus, the overall conclusion is that there is sufficient evidence that Bronopol exerts no endocrine activity.

Based on the EATS-mediated parameters there is no consistent evidence for adversity of Bronopol. Even though in some of the studies slight effects were observed, they were isolated, not reproducible in other studies, they were observed only at levels where Bronopol exerted marked systemic toxicity and they can clearly be considered to be of no biological relevance. Therefore, the overall conclusion is that there is no evidence for adversity regarding EAS-mediated parameters.

Table 94: Analysis of the integrated lines of evidence for potential estrogen-/ androgen-mediated or steroidogenesis-related activity and adversity.

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	Estrogen receptor	human	2	Hours	Uptake from the medium (*in vitro*)	-	No effect	Sufficient negative evidence for estrogen receptor activity	Overall sufficient negative evidence for EAS-related *in vitro* mechanistic activity	E
		yeast	72	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	80	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	8	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	8	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	8	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	2	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	8	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Estradiol level (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect	Supporting negative evidence for E-related endocrine activity		
	Androgen receptor	hamster	24	Hours	Uptake from the medium (*in vitro*)	-	No effect	Sufficient negative evidence for androgen receptor activity		A
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		hamster	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		hamster	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		human	8	Hours	Uptake from the medium (*in vitro*)	AC50 = 39.89 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM)			
		human	16	Hours	Uptake from the medium (*in vitro*)	AC50 = 39.76 µM	active above cytotoxicity limit (9.058 µM)			
		human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 89.28 µM	only highest conc. above baseline, less than 50% efficacy, active above cytotoxicity limit (9.058 µM)			
		human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 70.43 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM)			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 87.97 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM)			
		human	3	Hours	Uptake from the medium (*in vitro*)	AC50 = 24.59 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM)			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		human	3	Hours	Uptake from the medium (*in vitro*)	AC50 = 30.90 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM) t			
	Testosterone level (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect	Supporting negative evidence for A-related endocrine activity		
	CYP19	human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 76.26 µM	only highest conc. above baseline, active above cytotoxicity limit (9.058 µM)	Supporting negative evidence for S-related endocrine activity		S
	CYP enzyme inhibition/ induction	human	1	Hours	Uptake from the medium (*in vitro*)	AC50 = 8.28 µM	Change of activation level, less than 50% efficacy	No indication for effect on CYP enzymes (involved in steroid metabolism)	Most of the CYP enzymes tested with Bronopol are involved in the steroid metabolism (if at all steroid related), and may be only secondary relevant for steroidogenesis, but are included for informative purposes.	S
		human	0.5	Hours	Uptake from the medium (*in vitro*)	AC50 = 3.04 µM	Change of activation level			
		human	1	Hours	Uptake from the medium (*in vitro*)	AC50 = 1.74 µM	Change of activation level			
		human	0.75	Hours	Uptake from the medium (*in vitro*)	AC50 = 3.10 µM	Change of activation level			
		human	48	Hours	Uptake from the medium (*in vitro*)	AC50 = 68.85 µM	only highest conc above baseline, active above cytotoxicity limit			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							(9.058 µM)			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Estrone (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect	Supporting negative evidence for EAS-related activity		EAS
	Progesterone (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Cortisol (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	11-Deoxycorticosterone (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	11-Deoxycortisol (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	17-α-hydroxypregnelone (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	17-α-hydroxyprogesterone (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Androstenedione (*in vitro*)	human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
In vivo mechanistic	Vitellogenin (VTG) in males	*Pimephales promelas*	21	days	Uptake from water	-	No change in plasma VTG compared to control	Supporting evidence for EAS-related activity	Overall sufficient negative evidence for *in vivo* mechanistic	EAS
	Vitellogenin (VTG) in females	*Pimephales promelas*	21	days	Uptake from water	-	No change in plasma VTG			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							compared to control		activity	
	Phase I enzyme induction (*in vivo*)	rat	13	Weeks	Oral	-	No effect on hepatic CYP concentration, highest dose tested was 1000 ppm	Supporting evidence for EATS-related activity		EATS
EAS-mediated	Male 2nd sex characteristics in females	*Pimephales promelas*	21	days	Uptake from water	-	No treatment-related effect, highest dose tested 1 mg/L water	No evidence for endocrine adversity	Overall sufficient negative evidence for EAS-mediated adversity	A
	Testis weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No convincing positive evidence for endocrine activity/ adversity: single finding in one study, decrease of abs. weight confounded by reduced body weight		EAS
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant decrease of testis weight (abs.) in the mid dose group (highest evaluable dose group)			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Testis histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effects			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Epididymis weight	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for endocrine adversity		
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Epididymis histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Prostate weight	rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm	No evidence for endocrine adversity		
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Prostate histopathology (with seminal vesicles and coagulating glands)	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Seminal vesicles weight	rat	13	Weeks	Oral	-	Decreased weight of seminal vesicles in M (statistically significant only at lowest dose group) was considered of no toxicological relevance due to the absence of dose-dependency and full reversibility (not observed in recovery group)	No evidence for endocrine adversity		
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Seminal vesicles histopathology	rat	13	Weeks	Oral	-	No effect			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		rat	104	Weeks	Oral	-	No effect			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Coagulating gland histopathology	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500	No evidence for endocrine adversity		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mg/L water			
	Sperm morphology	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
	Sperm motility	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Sperm numbers	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Age at balanopreputial separation	rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
	Ovary weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No convincing positive evidence: single finding in one study, weight in the upper range for rats of this age and strain		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant increase of ovary weight (rel.) in the mid dose group (highest evaluable dose group)			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	13	Weeks	Oral	-	Group differences (stat. significantly decrease) were observed but not treatment-related, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Ovary histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Oviduct histopathology	rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm	No evidence for endocrine adversity		
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Uterus weight (with cervix)	rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d	No evidence for endocrine adversity		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0, F1 and F2, highest dose tested was 1500 mg/L water			
	Uterus histopathology (with cervix)	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested 200 mg/kg bw/d			
	Cervix histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for endocrine adversity		
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Vagina histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for endocrine adversity		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Spain 2-bromo-2-nitro-1,3-propanediol (Bronopol) 2, 11 & 12

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Mammary gland histopathology (female)	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for endocrine adversity		
		rat	13	Weeks	Oral	-	No effect			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Estrus cyclicity	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
	Age at vaginal opening	rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
	Specific gonad histopathology	Fish (*Pimephales promelas*)	21	days	Uptake from water	-	No treatment-related effect in female, highest dose tested 1 mg/L water	No evidence for endocrine adversity		

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Fish (*Pimephales promelas*)	21	days	Uptake from water	-	No treatment-related effect in male, highest dose tested 1 mg/L water			
	Male 2nd sex characteristics in males	Fish (*Pimephales promelas*)	21	days	Uptake from water	-	No treatment-related effect, highest dose tested 1 mg/L water	No evidence for endocrine adversity		

A.6.8.2. Assessment of the integrated lines of evidence and weight of evidence for potential thyroid-related adversity and activity of Bronopol

The lines of evidence have been assessed using the Excel template according to Appendix E of the guidance (ECHA/EFSA, 2018). The lines of evidence for potential thyroid disruption are assembled, integrated and assessed as shown in Table 95 below.

In vitro mechanistic studies were available for Bronopol regarding endocrine activity. Furthermore, the endocrine activity was investigated using in-silico methods. None of the models and mechanistic studies predicted or showed, respectively, a binding of Bronopol to or activity of Bronopol on TR. Furthermore, Bronopol had no effect on the thyroperoxidase. Thus, the overall conclusion is that there is sufficient evidence that Bronopol exerts no endocrine activity.

Based on the EATS-mediated parameters there is no consistent evidence for adversity of Bronopol in terms of the thyoid. Even though in some of the studies slight effects were observed, they were isolated, not reproducible in other studies, they were observed only at levels where bronopol exerted marked systemic toxicity and they can clearly be considered to be of no biological relevance. Therefore, the overall conclusion is that there is no evidence T-mediated adversity.

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Table 95: Analysis of the integrated lines of evidence for potential thyroid disruption

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	Thyroid receptor	human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect	No evidence for thyroid receptor transactivation	Overall sufficient negative evidence for T-mediated *in vitro* mechanistic activity	T
		rat	28	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		rat	28	Hours	Uptake from the medium (*in vitro*)	AC50 = 25.85 µM	Positive hit-call (antagonistic effect) only at cytotoxic concentrations (above cytotoxicity limit, 9.058 µM)			
		Human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect	No evidence for thyroid receptor binding		
		human	48	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	TSH receptor (*in vitro*)	human	0.5	Hours	Uptake from the medium (*in vitro*)	AC50 = 49.88 µM	active above cytotoxicity limit (9.058 µM)			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	AC50 = 47.06 µM	active only above cytotoxicity limit (9.058 µM)			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	0.5	Hours	Uptake from the	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
					medium (*in vitro*)					
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Thyroperoxidase activity (TPO) (*in vitro*)	rat	0.5	Hours	Uptake from the medium (*in vitro*)	-	No effect, highest conc. tested was 87.5 µM	No evidence for TPO inhibition *in vitro*		
	Na^+/I^- symporter activity (NIS) (*in vitro*)	human	2	Hours	Uptake from the medium (*in vitro*)		No effect, highest conc. tested was 100 µM	No evidence for NIS inhibition *in vitro*		
	Deiodinase 1 activity (DIO1) (*in vitro*)	human	3	Hours	Uptake from the medium (*in vitro*)		No effect, highest conc. tested was 200 µM	No evidence for DIOs inhibition *in vitro*		
	DIO2+3 activity (*in vitro*)	human	3	Hours	Uptake from the medium (*in vitro*)		No effect, highest conc. tested was 200 µM			
	Nuclear receptor (*in vitro*)	human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect	No evidence for receptor binding		
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 163.61 µM	Hit-call potentially confounded by overfitting, active above cytotoxicity limit (9.058 µM)			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	CAR nuclear receptor (*in vitro*)	human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Activation of thyrotropin-releasing hormone receptor	human	20	Hours	Uptake from the medium (*in vitro*)	-	No effect	No indication for inter-ference with thyrotropin releasing hormone receptor (TRHR)	Supporting negative evidence for T-mediated adversity	T
		human	20	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	T3 and T4 level	rat	13	Weeks	Oral	-	No effect in main study and recovery F, slight effect on T3 in recovery M still within historical controls, highest dose tested was 1000 ppm	No evidence for EATS-related activity	Overall sufficient negative evidence for *in vivo* mechanistic activity	T
	Thyroid-stimulating hormone level (TSH)	rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
	Phase I enzyme induction (*in vivo*)	rat	13	Weeks	Oral	-	No effect on hepatic CYP concentration, highest dose tested was 1000 ppm			EATS
T-mediated	Thyroid weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No convincing positive evidence: few	Overall sufficient negative	T

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	histopathological findings and rel. and/or abs. organ weight increase observed only in some studies; occur at systemic toxic doses; no associated histo-pathological findings	evidence for T-mediated adversity	
		rat	13	Weeks	Oral	80 mg/kg bw/day	Slight, but statistically significant increase of thyroid weight (rel.) in M of the mid dose group (highest evaluable dose group)			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Increased (abs. and rel.) thyroid weights in adult F0 F of the highest dose group (1500 mg/L water)			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Statistically significant increase of thyroid weight (rel.) in adult F1 M of the high dose group, related to the lower mean body weight of the adult F1 M			
	Thyroid histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	18	Weeks	Oral	500 mg/L water	Histopathological changes were observed only in F0 females (histopathology of thyroid not performed on F1 females due to absence of change in thyroid weight) thus interpreted as equivocal			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	HDL/LDL ratio (considered T-mediated only in combination with other thyroid endpoints)	rat	90	Days	Oral	-	No effect on Cholesterol level, highest dose tested was 1500 mg/L water	No evidence for T-mediated activity		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	90	Days	Oral	-	No effect on Cholesterol level, highest dose tested was 500 mg/L water			
	Developmental stage	Frog *(Xenopus laevis)*	3	weeks	Uptake from water	10 µg/L	Tadpoles exposed to highest dose showed more advanced developmental stage, however, as Bronopol was tested in a mixture with 22 other chemicals, the observed effects cannot be assigned to Bronopol alone	No convincing positive evidence: effect cannot be assigned to Bronopol as a mixture with 22 other chemicals was tested		

A.6.8.3. Assessment of the integrated lines of evidence of parameters sensitive to but not diagnostic of EATS modalities for Bronopol

The lines of evidence have been assessed using the Excel template according to Appendix E of the guidance (ECHA/EFSA, 2018). The lines of evidence for parameters sensitive to but not diagnostic of EATS modalities are assembled, integrated and assessed as shown in Table 96 below.

Parameters which are sensitive to but not diagnostic of EATS, revealed no specific effects but were confounded by systemic toxicity (*e.g.* reduced body weight). Therefore, no indication of potential ED-mediated effects for Bronopol are shown in the available data.

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Table 96: Analysis of the integrated lines of evidence for parameters sensitive to but not diagnostic of EATS modalities

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Sensitive to, but not diagnostic of, EATS	Brain weight	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No convincing positive evidence: no consistent findings; only in some studies, increase/decrease of (abs.) weight confounded by reduced body weight	Overall sufficient negative evidence for EATS-sensitive adversity	EATS
		rat	13	Weeks	Oral	80 mg/kg bw/day	Slight, but statistically significant increase of brain weight in M of the mid dose group (highest evaluable dose group)			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1 adults, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	1500 mg/L water	Decrease of absolute brain weight in F1 and F2 offspring at maximal dose of 1500 ppm (considered secondary to decreased body weight in F1 and F2 offspring)			
	Brain histopathology examination	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		mouse	80	Weeks	Dermal	-	No effect			
	Pituitary weight	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for endocrine adversity		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		dog	13	Weeks	Oral	-	No effect			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Pituitary histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Adrenals weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No convincing positive evidence: single finding in one study, decrease of		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant increase of adrenals weight (rel.) in M of the mid dose group (highest evaluable dose group)	abs. weight confounded by reduced body weight		
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Adrenals histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Time to mating	rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
	Gestation length	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d	No evidence for endocrine adversity		
		rat	21	Days	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, highest dose tested was 200 mg/kg bw/d			
	Fertility (mammals)	rat	19	Weeks	Oral	-	2 F failed to conceive due to 1 M that was found to have undescended testes, however not treatment-related, highest dose tested was 40	No evidence for endocrine adversity		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect on the percent pregnant animals (No. of females with visible implantations/total No. bred), highest dose tested was 30 mg/kg/d			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	Slightly lower mating and fertility incidences due to 4 F that didn't mate and 3 F that mated but did not litter; based on low incidence, considered spurious and unrelated to treatment, highest dose tested was 1500 mg/L water			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	Slightly decreased fertility index in F0 F (F1a mating only) of the high dose group, however not treatment-related, highest dose tested was 200 mg/kg bw/d			
	Number of ovarian follicles	rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d	No evidence for endocrine adversity		
	Number of implantations, corpora lutea	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d	No evidence for endocrine adversity		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
	Pre implantation loss	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d	No evidence for endocrine adversity		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
	Post implantation loss	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d	No convincing positive evidence: no specific effect on post-implantation loss, number		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rabbit	21	Days	Oral	-	Post implantation losses were significantly lower than in the control group in the groups treated at 5, 20 and 80 mg/kg bw/day (highest tested dose)	of live births, litter viability, and litter size observed		
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Increase in F1 adults, attributed to 3 dams (total of 27 animals), suggested possible relationship to maternal toxicity			
		rat	18	Weeks	Oral	-	No effect in F0, highest dose tested was 1500 mg/L water			
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Litter viability	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	21	Days	Oral	-	Slightly higher pub mortality was observed in both treatment groups at an unusually low incidence of pub death in the control group, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Decrease in F1 adults, 2 dams with total litter loss shortly after delivery (and 1 had post-implantation loss, total of 27 animals), suggested possible relationship to maternal toxicity			
		rat	18	Weeks	Oral	-	No effect in F0, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
	Litter size	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	21	Days	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect in F0, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	1500 mg/L water	Decrease of the litter size in F1 adults, attributed to 4 dams (total of 27 animals), suggested possible relationship to maternal toxicity			
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
	Number of live births	rat	21	Days	Oral	-	Total litter loss (1 at control, 2 at 20 and 1 at 40 mg/kg bw/d), not treatment-related, highest dose tested was 40			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Decrease of the number of live births in F1 adults, attributed to 4 dams (total of 27 animals), suggested possible relationship to maternal toxicity			
		rat	18	Weeks	Oral	-	No effect in F0, highest dose tested was 1500 mg/L water			
		rat	48	Days	Oral	-	Number of pubs born at 50 and 200 mg/kg bw/d were lower than control, but no dose-dependeny observed, highest dose tested was 200 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Pup survival index	rat	18	Weeks	Oral	-	No effect in F0, F1 and F2, highest dose tested was 1500 mg/L water	No evidence for endocrine adversity		
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No treatment-related effect (F0+F1). F1a mating: On PND 4, the survival index was slightly lower due to one litter that had 1 dead pup and 6 missing pubs on PND1. F1b mating: On PND 4, 7, 14 the pub survival index was slightly lower than control.			
	Dystocia	rat	18	Weeks	Oral	1500 mg/L water	Increase in F0 adults, seen in 2 dams (total of 27 animals); gross and histopathologic findings did not reveal specific cause, detailed examination suggested possible relationship to maternal toxicity	No convincing positive evidence: no indication for clear specific effect		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Sex ratio	rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d	No evidence for endocrine adversity		
		rat	18	Weeks	Oral	-	No effect in F0 and F1, highest dose tested was 1500 mg/L water			
	Numbers of embryonic or foetal deaths and viable foetuses	rat	19	Weeks	Oral	-	No effect, except for total liter loss in 1 animal of the high dose group, highest dose tested was 40 mg/kg bw/d	No convincing positive evidence: only one animal in one study affected		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
	Litter/pup weight	rat	19	Weeks	Oral	-	No effect, except for slightly decreased litter pub weights in the high dose group at birth and PND1. At PND21 litter weight	No convincing positive evidence: no indication for clear specific effect		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							was comparable to control, highest dose tested was 40 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	21	Days	Oral	-	No effect, except for litter and pub weight slightly below the control in both treatment groups from PND 12-21, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Decrease of the litter/pup weight in F1 offspring, at PND 7 and 14 (transient effect, no significant difference at PNDs 1, 4 or 21 and the partial recovery of body weight by PND 21 correlated with increased water consumption and body weight gain of lactating F)			
		rat	18	Weeks	Oral	-	No effect in F2 offspring, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	48	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
	Functional observation battery	rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm	No evidence of any impact on behavior or activity		
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Motor activity	rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
	Fetal development	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d	No convincing positive evidence: impairment of fetal weight occured at systmic toxic dose (secondary effect)		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			
		rabbit	21	Days	Oral	80 mg/kg bw/day	Statistically significant reduced fetal weight and increased incidence of runted fetuses in the high dose group			
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
	Presence of anomalies	rat	19	Weeks	Oral	-	No effect, highest dose tested was 40	No convincing positive		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	(external, visceral, skeletal						mg/kg bw/d	evidence: no indication for clear specific effect, effects occurred at systemic toxic doses		
		rabbit	12	Days	Oral	-	No effect, highest dose tested was 80/160 mg/kg bw/d			
		rabbit	21	Days	Oral	80 mg/kg bw/day	General retarded skeletal ossification, increased incidence of fetuses with non-ossification of the fore- and hind-limb epiphyses in fetuses of the high dose group			
		rat	14	Days	Oral	-	No effect, highest dose tested was 100 mg/kg bw/d			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rabbit	21	Days	Oral	30 mg/kg bw/day	Increase of axial-skeletal malformations in fetuses, at PND 7 and 14; 3 of the 4 high dose litters producing fetuses with axial skeleton malformations were among those with the greatest degree of maternal toxicity (large body weight loss GD 7-10 or GD 7-13 and reduced feed consumption) suggesting relationship to maternal toxicity			
	Length (fish)	*Oncorhynchus mykiss*	49	Days	Uptake from water	-	No treatment-related effect, highest dose tested was 40 mg/L water	No evidence for endocrine adversity		
	Body weight (fish)	*Pimephales promelas*	21	days	Uptake from water	-	No reduction in body weight of female compared to control, highest dose tested was 1 mg/L water			
		Pimephales promelas	21	days	Uptake from water	-	No reduction in body weight of male compared to control, highest			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							dose tested was 1 mg/L water			
		Oncorhynchus mykiss	49	Days	Uptake from water	-	No treatment-related effect, highest dose tested was 40 mg/L water			
	Behaviour (fish)	*Oncorhynchus mykiss*	49	Days	Uptake from water	-	No treatment-related effect, highest dose tested was 40 mg/L water			
	Reproduction (fecundity, fertility)	*Pimephales promelas*	21	days	Uptake from water	-	No increase in fecundity compared to control, highest dose tested was 1 mg/L water			
	Body weight (amphibian)	Frog *(Xenopus laevis)*	3	weeks	Uptake from water	10 µg/L	Tadpoles exposed to highest dose showed higher variation in body weight, however, as Bronopol was tested in a mixture with 22 other chemicals, the observed effects cannot be assigned to Bronopol alone			
	Body weight (bird)	Bobwhite quail	5	days	Oral	-	No effect		Overall sufficient	EATS

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Mallard duck	5	days	Oral	5000 ppm	A treatment-related reduction in mean body weight increase over 5 days was observed in the two highest treatments.		negative evidence for EATS-sensitive adversity	

A.6.8.4. Assessment of the integrated lines of evidence of target organ and general toxicity of Bronopol

The lines of evidence have been assessed using the Excel template according to Appendix E of the guidance (ECHA/EFSA, 2018). The lines of evidence target organ and general toxicity are assembled, integrated and assessed as shown in Table 97 below.

Bronopol showed due to its irritant nature some effects on the stomach as a first point of contact organ in oral studies. Furthermore, several subchronic or chronic studies identify kidney toxicity including histopathological correlates and indicating kidney impairment. However, for both effects clear NOAELs can be derived. Besides there are some subchronic or chronic studies indicating sporadic liver changes, but without histopathological correlate. Moreover, significant effects on body weight, food consumption, mortality and clinical signs were observed at high concentrations and at subchronic or chronic exposure. Minor effects on haematology were observed without indication of specific organ toxicity. As a conclusion, clear effects of general toxicity were observed at high concentrations and long-term exposure.

Table 97: Analysis of the integrated lines of evidence of target organ and general toxicity

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Target organ toxicity	Kidney weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	Consistent evidence of dose dependent effects on kidney weight	Overall positive evidence for kidney toxicity	NA
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d	and histopathology ; clear NOAEL; indicative for kidney impairment		
		rat	90	Days	Oral	1500 mg/L water	Rel. Kidney weight was increased in M of the high dose group			
		rat	13	Weeks	Oral	1000 ppm	Slightly increased rel. kidney weight in high dose F and a minimal trend for increased kidney weights in high dose M			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	104	Weeks	Oral	160 mg/kg bw/day	Statistically significant increase of kidney weight (rel.) in M+F of the high dose group, without histopathological correlate			
		rat	18	Weeks	Oral	1500 mg/L water	Kidney weight (abs. and rel.) was increased in adult F0 M+F of the high			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							dose group			
		rat	18	Weeks	Oral	1500 mg/L water	Kidney weight (rel.) was increased in adult F1 M+F of the high dose group			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Decreased kidney weights (abs.) in F2 offspring F of the high dose group			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	25 mg/kg bw/day	Dose related increase of kidney weight in adult F0 F; statstically significant increase of kidney weight (rel.) in adult F1 M of the high dose group, related to lower mean body weight of the adult F1 M.			
	Kidney histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	20 mg/kg bw/day	Distended renal tubules with infiltrates in the adjacent interstitial tissue (1 M of low dose; 1 M of mid dose), dilated tubules with infiltrates in the corticomedullary junction (1 M of low dose; 1 M of mid dose)			
		rat	90	Days	Oral	1500 mg/L water	Slight nephropathy in M only, slight bilateral multifocal tubular epithelial degeneration with regeneration, bilateral multifocal dilatation of medullary tubules and slight subacute to chronic multifocal interstitial inflammation			
		rat	13	Weeks	Oral	1000 ppm	Increased incidence of basophilic tubules (F), hyaline casts at the corticomedullary junction (M) and in the loops of Henle			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							at the renal papilla (M+F) at the highest dose group			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	160 mg/kg bw/day	Increased incidence of chronic glomerulonephropathy in M+F of the high dose group			
		rat	18	Weeks	Oral	1500 mg/L water	Adult F0 M+F of the high dose group showed very slight or slight, multifocal tubular degeneration with regeneration and very slight or slight, multifocal tubular dilatation; very slight or slight multifocal, subacute to chronic interstitial inflammation			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	500 mg/L water	Adult F1 M of the mid dose and adult F1 M+F of the high dose group showed very slight or slight, multifocal tubular degeneration with regeneration and very slight or slight, multifocal tubular dilatation			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Granular-appearing kidneys and a progressive nephropathy and constituent tubular dilatation in F0 F of the high dose group; Spontaneous nephropathy observed in all treatment groups of adult F1			
	Liver weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	Only high dose effect on sporadic weight changes; no clear picture of		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant increase of liver weight (rel.) in F of the mid dose group (highest evaluable dose group)	the affected sex; without histopathological correlate; clear NOAEL		
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	20 mg/kg bw/day	Statistically significant increase of liver weight (rel.) in M of the high dose group due to one animal			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 30 mg/kg bw/d			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Decreased liver weights (abs.) in adult F1 M and in M+F F2 offspring of the high dose group			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Liver histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	18	Weeks	Oral	1500 mg/L water	Adult F0 F showed very slight-slight multifocal hepatocyte vacuolization, consistent with fatty change			
		rat	18	Weeks	Oral	1500 mg/L water	Adult F1 F showed very slight-slight multifocal hepatocyte vacuolization, consistent with fatty change			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect, highest dose tested was 200 mg/kg bw/d			
	Thymus weight	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	High dose effect in two studies in one sex; no consistent evidence for thymus impairment		
		rat	13	Weeks	Oral	80 mg/kg bw/day	Slight, but statistically significant decrease of thymus weight (rel.) in M of the high dose group			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	1500 mg/L water	Decreased thymus weight (abs.) in F2 offspring M of the high dose group			
	Thymus histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
	Heart weight	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for heart impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
			68							
	Heart histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Spleen weight	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	Only high dose effect in a single study, no clear evidence for		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							160 mg/kg bw/d	spleen impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	20 mg/kg bw/day	Statistically significant increase of spleen weight (rel.) in M of the high dose group due to one animal			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	18	Weeks	Oral	-	No effect, highest dose tested was 1500 mg/L water			
	Spleen histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Stomach histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	Concentration-related effect at the site of first contact due to irritating properties of Bronopol; clear NOAEL		
		dog	28	Days	Oral	500 mg/L water	Slight multifocal hypertrophy of mucous cells in M of the high dose group			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rabbit	12	Days	Oral	80/160 mg/kg bw/day	Haemorrhage and ulceration of gastric mucosa were observed in all animals (F) of the high dose group			
		rat	14	Days	Oral	100 mg/kg bw/day	In 2/3 F killed ulceration of gastric mucosa was observed			
		rat	104	Weeks	Oral	160 mg/kg bw/day	Epithelial hyperplasia associated with ulceration of gastric mucosa, occasional squamous cell papilloma, dilatation of gastric lymph nodes sinusoids was observed in M+F of			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							the high dose gorup			
		rat	18	Weeks	Oral	1500 mg/L water	Adult F0 F showed multifocal erosions in the glandular mucosa, focal ulcers and slight acute multifocal inflammation of the glandular submucosa			
		rat	18	Weeks	Oral	1500 mg/L water	Adult F1 F showed multifocal erosions in the glandular mucosa and slight acute multifocal inflammation of the glandular submucosa			
		rat	48	Days	Oral	-	No effect, except for ulcers and thickened mucosa in the non-glandular stomach and bloody gastrointestinal contents in 1 M sacrificed moribund, highest			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							dose tested was 200 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Lymph nodes histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for impairment of lymph nodes		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
	Salivary glands histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for salivary gland impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	40 mg/kg bw/day	Squamous metaplasia in the ducts associated with minimal mixed or chronic inflammatory cell infiltration and groups of atrophic acini in M of the high and mid dose group and in F of the high dose group. Affected glands dilated and minimal epithelial hyperplasia noted. Effects are			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							considered to be of no toxicological significance.			
	Aorta histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for aorta impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Bone histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for bone impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Bone marrow	dog	28	Days	Oral	-	No effect, highest	No evidence		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	histopathology						dose tested was 500 mg/L water	for bone marrow impairment		
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
	Eyes histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for eye impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
	Gall bladder histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for gall bladder impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			

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Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Lung histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for lung impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Pancreas histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was	No evidence for impairment		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							0.5 %	of pancreas		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
	Peripheral nerve histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for impairment of peripheral nerve		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Skeletal muscle histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for skeletal muscle impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Skin histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for impairment of skin		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
	Small and large intestines histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for impairment of small and large intestines		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	No effect in F0, F1, F2, highest dose tested was 200 mg/kg bw/d			
	Spinal cord histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for impairment of spinal cord		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
	Trachea histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for trachea impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Urinary bladder histopathology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	No evidence for urinary bladder impairment		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			
	Oesophagus histopathology	dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water	No evidence for oesophagus impairment		
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
Systemic toxicity	Mortality	Fish *(Pimephales promelas)*	21	days	Uptake from water	-	No effect on female, highest dose tested was 1 mg/L water	Dose-related effects with a clear NOAEL; indicative for	Overall clear evidence for systemic toxicity	

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Fish *(Pimephales promelas)*	21	days	Uptake from water	-	No effect on male, highest dose tested was 1 mg/L water	systemic toxicity		
		rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %			
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	All animals of the high dose and about half of the mid dose group died or were killed for humane reasons			
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	19	Weeks	Oral	-	No effect, 1 M and 2 F of the high dose and 1 M and 1 F of the low dose died during the study, highest			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							dose tested was 40 mg/kg bw/d			
		rabbit	12	Days	Oral	80/160 mg/kg bw/day	Increased mortality was observed in F of the high dose group (mid dose: GD7-13; high dose: GD 14-16).			
		rabbit	21	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rat	14	Days	Oral	100 mg/kg bw/day	Three F of the high dose group were killed in extremis			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rabbit	21	Days	Oral	-	No effect, except for mortality of 2 F at 10 mg/kg bw/day (gavage error identified in 1/2) and 1 F at the high dose group, highest dose tested was 30 mg/kg bw/d			
		rat	104	Weeks	Oral	160 mg/kg bw/day	Statistically significant increase of mortality in M+F of the high dose			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							group			
		mouse	80	Weeks	Dermal	-	Marginally inferior survival rate, not treatment-related, highest dose tested was 0.5%			
		Fish (*Oncorhynchus mykiss*)	49	Days	Uptake from water	40 mg/L water	Mortality was increased in the highest dose group			
		Fish (*Oncorhynchus mykiss*)	28	Days	Uptake from water	10 mg/L water	Mortality was increased in the two highest treatments			
		Fish (*Oncorhynchus mykiss*)	30	minutes/day	Uptake from water	-	No effect, highest dose tested was 20 mg/L water			
		Fish (*Oncorhynchus mykiss*)	30	minutes/day	Uptake from water	-	One fish died in highest dose group, natural variation, highest dose tested was 100 ppm			
		Fish (*Plecoglossus altivelis*)	30	minutes/day	Uptake from water	-	One fish died in each of the two dose groups, natural variation, highest dose tested was 100 ppm			
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water	Cumulative mortality for eggs treated with Bronopol was			
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water	decreased, following daily application of formulated product containing Bronopol for 30 min/day until majority of eggs had hatched (after 61-65 days)			
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		Fish (*Salmo trutta*)	30	minutes/day	Uptake from water	50 mg/L water				
		rat	21	Days	Oral	-	No effect, except for 1 dead animal at the low and 1 dead animal at the high dose group (that died during the post partum period), highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Mortality was increased in F of the high dose group, and included treatment-related spontaneous death			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							in 2 F with dystocia			
		rat	48	Days	Oral	-	No effect, except for 1 M of the high dose group sacrificed moribund, highest dose tested was 200 mg/kg bw/d			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	-	8 animals of F0 (1 M/7 F, 1 control, 1 low dose, 6 high dose) and 3 animals of F1 (3 F, one per treatment group) died. Mortality is considered not treatment-related due to absence of consistent trend of necropsy findings, highest dose tested was 200 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Bobwhite quail	5	days	Oral	1000 ppm	Increase in mortality with a dose-response relationship, starting from 20% mortality at 1000 ppm up to 90% mortality at the highest treatment of 10,000 ppm			
		Mallard duck	5	days	Oral	-	No effect			
	Clinical signs	rabbit	21	Days	Dermal	0.2%	Slight to well defined erythema in the low dose group progressed to moderate erythema in almost all M+F of the high dose group	Dose-related effects with a clear NOAEL; indicative for systemic toxicity		
		dog	28	Days	Oral	-	No effect, except soft feces in treated M, not considered treatment related, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	M+F of the mid and high dose group showed respiratory distress and abdominal distension			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		dog	13	Weeks	Oral	20 mg/kg bw/day	M+F of the high dose group were vomiting			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	19	Weeks	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rabbit	12	Days	Oral	80/160 mg/kg bw/day	Reduced number and size of fecal pellets in animals of the high dose group			
		rabbit	21	Days	Oral	80 mg/kg bw/day	Reduced number and size of fecal pellets in animals of the high dose group			
		rat	14	Days	Oral	100 mg/kg bw/day	Noisy and laboured respiration, hunched posture in one animal killed in extremis in the high dose group			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rabbit	21	Days	Oral	30 mg/kg bw/day	Decreased fecal output in high dose F			
		rat	104	Weeks	Oral	160 mg/kg bw/day	M+F of the high dose group showed reduced grooming activity			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		mouse	80	Weeks	Dermal	0.5%	Minimal hair loss around treatment area (wk 1-3), signs of skin irritation at the high dose animals			
		rat	21	Days	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Red perinasal soiling, cold to touch, shallow breathing, and/or decreased activity of adult F0 F in the high dose group			
		rat	18	Weeks	Oral	1500 mg/L water	Red perinasal soiling, red vulvar discharge, and/or perineal soiling of the adult F1 F in the high dose group			
		rat	48	Days	Oral	-	Hair loss, malaligned incisors, and dried material around the eyes and/or nose, however not treatment-related, highest dose teste was 200 mg/kg bw/d			
	Food consumption	rabbit	21	Days	Dermal	-	No effects in M and no effect in F,	Dose-related effects with a		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							except depression of food consumption in 2/5 at 0.2% and 1/5 at 0.5%, highest dose tested was 0.5%	clear NOAEL; indicative for systemic toxicity		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant reduced food consumption in the high and mid dose group in week 1. Food consumption recovered from 2nd week on in the mid dose group.			
		rat	90	Days	Oral	1500 mg/L water	Decreased food consumption in M of the high dose group			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rabbit	12	Days	Oral	80/160 mg/kg bw/day	Decreased food consumption in individual F of the high dose group (mid dose: GD7-13; high dose: GD 14-16).			
		rabbit	21	Days	Oral	80 mg/kg bw/day	Decreased food consumption (stat. significant at GD 7-11) in F of the high dose group			
		rat	14	Days	Oral	100 mg/kg bw/day	Decreased food consumption in all F of the high dose group (GD 6-9)			
		rat	14	Days	Oral	-	No effect, highest dose tested was 80 mg/kg bw/d			
		rabbit	21	Days	Oral	-	Not statistically identified, but animals of the high dose group had sporadic occurrences of slightly reduced mean feed consumption, highest dose tested was 30 mg/kg bw/d			

Spain 2-bromo-2-nitro-1,3-propanediol (Bronopol) 2, 11 & 12

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	104	Weeks	Oral	40 mg/kg bw/day	Statistically significant decrease of food consumption in M of the mid and high dose group. Reduction in water intake and associated reduction in urine volume in wk 25, 52, 103.			
		rat	104	Weeks	Oral	-	Food consumption of F similar to control during the first 78 weeks of treatment; thereafter, a slight reduction in food consumption was observed, highest dose tested was 160 mg/kg bw/d			
		mouse	80	Weeks	Dermal	-	No effect, highest dose tested was 0.5 %			
		rat	21	Days	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	500 mg/L water	Decreased food consumption in adult F0 F of the mid and high dose group and M of the high dose group			

Spain 2-bromo-2-nitro-1,3-propanediol (Bronopol) 2, 11 & 12

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	1500 mg/L water	Decreased food consumption in adult F1 M+F of the high dose group			
		rat	48	Days	Oral	200 mg/kg bw/day	Reduced food consumption was found in M+F of the high dose group (wk 1). Water consumption was reduced in all M (throughout the study) and in some F (until wk 2)			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Slightly to markedly temporary reduced food consumption was observed throughout the whole study in M+F of the high dose group and in particular during gestation (F1) and lactation (F0 and F1) period. Water consumption of treated M+F was less than control throughout the whole study period.			
	Body weight	rabbit	21	Days	Dermal	-	No effect, highest	Dose-related		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							dose tested was 0.5 %	effect on body weight with a clear NOAEL; indicative of systemic toxicity and that MTD was reached		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	80 mg/kg bw/day	Statistically significant reduced body weight in M of the high group. Body weight recovered from 2nd week on in the mid dose group.			
		rat	90	Days	Oral	1500 mg/L water	Decrease of body weight gain (by 4.4%, non-sign.) in high dose M, not considered toxicologically relevant			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 1000 ppm			
		dog	13	Weeks	Oral	-	No effect, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	19	Weeks	Oral	40 mg/kg bw/day	Decreased body weight gain in M of the high dose group			

Spain 2-bromo-2-nitro-1,3-propanediol (Bronopol) 2, 11 & 12

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rabbit	12	Days	Oral	80/160 mg/kg bw/day	Reduced body weight and body weight gain in individual F of the high dose group			
		rabbit	21	Days	Oral	80 mg/kg bw/day	Reduced body weight and body weight gain (GD 7-11) in F of the high dose group			
		rat	14	Days	Oral	30 mg/kg bw/day	Statistically significant reduced body weight and body weight gain in F at the mid and high dose level			
		rat	14	Days	Oral	80 mg/kg bw/day	Decrease in body weight gain (GD 6-7) in F of the high dose group			
		rabbit	21	Days	Oral	30 mg/kg bw/day	Although not statistically identified, 1/2 of F had mean body weight loss at start of treatment and slightly lower gains over the entire treatment period that was considered treatment related			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	104	Weeks	Oral	40 mg/kg bw/day	Statistically significant decrease of body weight gain in M of the mid and high dose group			
		rat	104	Weeks	Oral	160 mg/kg bw/day	Statistically significant decrease of body weight gain in F of the high dose group			
		mouse	80	Weeks	Dermal	0.5%	Statistically significant decreased body weight gain (wk 26-52) in M of the high dose group			
		rat	21	Days	Oral	-	No effect, highest dose tested was 40 mg/kg bw/d			
		rat	18	Weeks	Oral	1500 mg/L water	Decreased weight gain of high dose F during GD14-21 and increased weight gain during lactation			
		rat	18	Weeks	Oral	1500 mg/L water	Decreased body weight gain of high dose F during GD 14-21 and increased weight gain during lactation			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	48	Days	Oral	25 mg/kg bw/day	Decreased body weight and body weight gain in M (wk 1-8) of all dose groups			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Statistically significant reduced body weight in adult F0 F (wk 20-21;30-31) and in adult F1 M+F of the high dose group. Maternal body weight gain was slightly (F0) and notably (F1) less than control (GD 0-20).			
		rat	P: 137, F1: 177-191, F2: 54-68	Days	Oral	200 mg/kg bw/day	Statistically significant decreased pup weight at PND 7 and 14 (F1a), at PND 4-21 (F1b+F2a) and at PND 0-21 (F2b)			
	Clinical chemistry and haematology	rabbit	21	Days	Dermal	-	No effect, highest dose tested was 0.5 %	Sporadic changes; dose-dependent with a clear NOAEL		
		dog	28	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	13	Weeks	Oral	-	No effect, highest dose tested was 160 mg/kg bw/d			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	90	Days	Oral	-	No effect, highest dose tested was 1500 mg/L water			
		dog	13	Weeks	Oral	-	No effect, except total white cell count although within normal limits was significantly lower in dogs receiving 8 and 20 mg/kg/day compared to control, highest dose tested was 20 mg/kg bw/d			
		dog	90	Days	Oral	-	No effect, highest dose tested was 500 mg/L water			
		rat	104	Weeks	Oral	40 mg/kg bw/day	Urine repeatedly positive for haemoglobin in M+F of the high dose group and in M of the mid dose group			
	Kidney histopathology (fish)	*Pimephales promelas*	21	days	Uptake from water	0.11 mg/L water	Low occurrence of increased immature nephrons (minimal) in males at all concentrations, however, it is not unusual to find immature	No clear evidence of systemic toxicity		

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							nephrons in the kidney of adult fish and the relationship to treatment for that particular finding is considered uncertain due to the absence of additional effects suggestive of Bronopol-induced renal toxicity			
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low occurrence of increased immature nephrons (minimal) in females at the highest concentration, however, it is not unusual to find immature nephrons in the kidney of adult fish and the relationship to treatment for that particular finding is considered uncertain due to the absence of additional effects suggestive of			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							Bronopol-induced renal toxicity			
	Liver histopathology (fish)	*Pimephales promelas*	21	days	Uptake from water	1 mg/L water	Low prevalence of cholangiocarcinoma in the liver of one female exposed to the highest concentration, but not clinically appreciable and not considered endocrine-mediated			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low prevalence of cholangiofibrosis (moderate) in females at the highest concentration, but not clinically appreciable and not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low prevalence of foci of cellular alteration in one female exposed to the highest concentration, but not clinically appreciable and not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	-	No effect, no single cell necrosis observed			
		Pimephales promelas	21	days	Uptake from water	0.33 mg/L water	Low frequencies of focal necrosis (minimal-mild) in females of all treatments except the lowest one, but not clinically appreciable and not considered endocrine-			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mediated			
		Pimephales promelas	21	days	Uptake from water	0.11 mg/L water	Low freuencies of cystic degeneration (minimal-moderate) in females at all concentrations, but not clinically appreciable and not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	-	No effect, observed hepatic basophilia in livers of females were not treatment-related as they were also observed in the control			
		Pimephales promelas	21	days	Uptake from water	-	No effect, observed vacuolation was not treatment-related			
		Pimephales promelas	21	days	Uptake from water	-	No effect, no cholangiocarcinoma observed			
		Pimephales promelas	21	days	Uptake from water	0.11 mg/L water	Low prevalence of cholangiofibrosis (mild-severe) in males at all concentrations, but not clinically appreciable and			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low prevalence of foci of cellular alteration in one male exposed to the highest concentration, but not clinically appreciable and not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low frequencies of single cell necrosis (minimal) in males at the highest concentration, but not clinically appreciable and not considered endocrine-mediated			
		Pimephales promelas	21	days	Uptake from water	-	No effect, no focal necrosis observed			
		Pimephales promelas	21	days	Uptake from water	1 mg/L water	Low freuencies of cystic degeneration (minimal) in males at the highest concentrations, but not clinically appreciable and not considered endocrine-			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							mediated			
		Pimephales promelas	21	days	Uptake from water	-	No effect, no basophilia in males observed			
		Pimephales promelas	21	days	Uptake from water	-	No effect, observed vacuolation was not treatment-related			
	Mortality (amphibian)	Frog *(Xenopus laevis)*	3	weeks	Uptake from water	5 µg/L	Survival was monitored for 3 weeks after end of exposure period and significantly decreased for the two highest dose groups, however, as Bronopol was tested in a mixture with 22 other chemicals, the observed effects cannot be assigned to Bronopol alone	Dose-related effects with a clear NOAEL; indicative for systemic toxicity		
In life observation	neurobehavioral parameters	dog	28	Days	Oral	-	No effect	No relevant effect besides signs of local irritation and decreased water consumption in some but not all available	Information included for informative purposes only, as there is no direct link to an ED activity for this assay endpoint.	NA
	ophthalmoscopic examinations	dog	28	Days	Oral	-	No effect			
	water consumption	dog	28	Days	Oral	-	No effect			
		rat	104	Weeks	Oral	40 mg/kg bw/day	Decreased water consumption observed			

Spain	2-bromo-2-nitro-1,3-propanediol (Bronopol)	2, 11 & 12	

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
		rat	18	Weeks	Oral	500 mg/L water	Decreased water consumption observed	studies (potentially related to palatability issues)		
		rat	18	Weeks	Oral	1500 mg/L water	Decreased water consumption observed			
	Body weight	Fish (*Onchorhynchus mykiss*)	28	Days	Uptake from water	-	No effect on specific growth rate based on body wet weight			
Organ histopathology	Nasal cavity	dog	28	Days	Oral	50 mg/L water	Very slight, multifocal, subacute to chronic inflammation of the nasal mucosa			
	Preputial/clitorial gland histopathology	rat	18	Weeks	Oral	-	No effect			
		rat	18	Weeks	Oral	-	No effect			
		rat	90	Days	Oral	-	No effect			
Reproductive	Uterus weight	rabbit	21	Days	Oral	-	No, effect, no change in Gravid Uterus weight			
Abnormalities	Tumour incidence	rat	104	Weeks	Oral	-	No effect			
		mouse	80	Weeks	Dermal		No effect			
In vitro mechanistic	Cytotoxicity assay	human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect	No relevant effects observed	Assays included for informative purposes only, as there is no	NA
		human	24	Hours	Uptake from the medium (*in vitro*)	AC50 = 80.01 µM	only highest conc. above baseline,			

Effect classification	Effect target	Species	Duration of exposure		Route of administration	Lowest Effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
							less than 50 % efficacy		link to an ED activity for this assay endpoint.	
		human	2	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
	Level of phosphorylated H3 histone, family 3A protein (H3F3A)	human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect	No relevant effects observed	Assays included for informative purposes only, as there is no direct link to an ED activity for this assay endpoint.	NA
		human	24	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	72	Hours	Uptake from the medium (*in vitro*)	-	No effect			
		human	72	Hours	Uptake from the medium (*in vitro*)	-	No effect			

A.6.9. OVERALL ASSESSMENT

A.6.9.1. Human health

A.6.9.1.1. Analysis of the evidence

As described in chapter 3.4.1 of the guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (further referred to as "ED Guidance document", ECHA/EFSA 2018), the EAS-mediated adversity with regard to humans and mammals (as non-target organisms) is sufficiently investigated when all "EAS-mediated" parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation (OECD, 2012b)) or a two-generation reproductive toxicity study (OECD TG 416) is available. A two-generation study conducted according to OECD TG 416 is available for Bronopol [[redacted] 2008]. The two-generation reproductive toxicity study addressed general toxicity, fertility and reproduction. The maximal test concentration induced systemic toxicity (reduction in body weight gain, increased mortality and also clinical signs) and also histopathological changes in target organs (stomach, liver and kidney) and thus fulfils the regulatory demands. In this study, no adverse effect of Bronopol on any EAS-related parameter was found in the absence of maternal toxicity. This conclusion is further supported by another two-generation reproductive toxicity study for Bronopol, indicating no evidence of any specific EAS-mediated adversity [[redacted] 1987].

More specifically, the endpoints providing information on EAS-mediated adversity can be summarized as effects on weights and histopathology of the relevant organs, as well as parameters for fertility and fetal development (please refer to the WoE for potential EAS-mediated adversity and activity below for a comprehensive summary). EAS-relevant adverse effects have been assessed in two sub-acute toxicity study, five sub-chronic toxicity studies, a one-generation reproductive toxicity study, three pre-natal developmental toxicity study as well as the two above mentioned two-generation reproduction toxicity studies with Bronopol. Furthermore, two combined chronic toxicity and carcinogenicity studies on Bronopol [[redacted] 1976, as reported by [redacted] *et al.* 1987 and US EPA RED 1995].

In more detail, the sub-chronic toxicity of Bronopol has been assessed in three studies in rats, as well as two studies in dogs after oral exposure. Rats were exposed to up to 160 mg/kg bw/d [[redacted] 1973], up to 1000 ppm [[redacted] 2001] or up to 1500 ppm [[redacted] 2006] for 90 days. These maximal concentrations were found sufficient to induce some target organ toxicity in kidneys; furthermore, increased mortality was observed at 160 mg/kg bw/d (LD100 at 160 mg/kg bw/d and 30-45% mortality at next lower dose 80 mg/kg bw/d) and further signs of systemic toxicity were found at 1500 ppm (decreased body weight gain, decreased food consumption), demonstrating that the chosen highest dose levels can be considered as maximal tolerable doses (MTD), or even beyond. In few of the available repeated dose studies, changes in relative organ weights (*e.g.* testis, adrenals, thyroid) were observed above the respective NOAELs (Table 1; Level 4). However, as already emphasized, those effects did not occur segregated, *i.e.* all (relative) organ weight changes were linked to systemic adverse effects, *e.g.* changes in body weight. Furthermore, such changes were within historical controls, and/or showed no dose-response relationship, and/or showed no histopathological correlate, and/or were not consistently reproducible comparing the different available repeated dose toxicity studies. No treatment-related histopathological changes were observed in any endocrine-related organ examined that may have been suggestive of any endocrine mediated effect.

In dogs, exposure to up to 20 mg/kg bw/d or 500 mg/L of Bronopol for 90 days did not induce any adverse effect on EAS-related organs. Furthermore, in rabbits repeated exposure to 0.5% Bronopol dermally applied did not reveal any EAS-mediated adversity,

Thus, it can be concluded that no specific and no segregated EAS-specific effects were observed in available repeated dose studies that would suggest a specific Bronopol-induced endocrine disrupting effect. Furthermore, with regard to the available *in vitro* data

it is very likely that the above-mentioned findings are not a consequence of an endocrine mode of action.

Information on the impact of Bronopol on fertility and reproductive parameters as well as developmental toxicity, also indicative of EAS-modalities, is provided in the two-generation reproduction toxicity studies and a peri- and postnatal study in rats as well as the pre-natal developmental toxicity studies in rats and rabbits. In one of the available prenatal developmental toxicity studies in rabbits, a marginal increase in the overall incidence of axial skeletal malformations (7 fetuses from 4 litters) at the high dose was attributed to maternal toxicity [[redacted] 2007]. Also, in the second pre-natal developmental toxicity study in rabbits [[redacted] 1991a] a general retardation of the foetal skeletal ossification and growth was observed at maternal toxic doses (80 mg/kg bw/d) which were >10fold above the chronic NOAEL. Those retardations correlated with marked maternal toxicity, such as decreased body weight and body weight gain, decreased food and water consumption and a reduction in number of faecal pellets and size,. In the more recent two-generation reproductive toxicity study according to OECD TG 416, reproductive effects were attributable to 6 high dose dams, several of which exhibited clear signs of maternal toxicity in late gestation [[redacted] 2008]. The observed effects in the developmental and reproductive toxicity studies were considered secondary to maternal toxicity and do not represent EATS-mediated effects. This is further supported by the other available two-generation study [[redacted] 1987], at which treatment-related effects referring to systemic toxicity were seen at all tested doses of Bronopol but were particularly pronounced at the highest dose tested, which was about 30fold higher than the chronic NOAEL for systemic toxicity. Regarding reproductive or litter parameters, some abnormalities but no significant treatment-related effects were reported at the highest dose; in fact, the findings reported at the highest test dose of 200 mg/kg bw rather resulted from the high systemic toxicity observed at this dose level than indicating reproductive toxicity. In conclusion, in guideline-conform prenatal develop¬men¬tal toxicity and one- or two-generation studies, Bronopol exposure had no effects on reproductive parameters and no effect on the developing foetuses in rats or rabbits in the absence of maternal toxicity.

Taken together, the available information provide no robust evidence for endocrine activity as well as adversity with regard to the estrogen, androgen, and steroidogenic modalities. Beside the absence of consistent evidence in the relevant organs, no relevant changes in potentially sensitive organs and reproductive parameters have been observed in the available studies (see summaries in table 98 and table 99 below).

Table 98: WoE for potential EAS-mediated adversity of Bronopol

- No consistent indications of **EAS-mediated adversity** of Bronopol as follows:
  - No effect on age at **vaginal opening** in F1 offspring in a two-generation reproduction toxicity study after oral exposure up to 1500 ppm Bronopol for 18 weeks
  - No effect on **estrus cyclicity** in F0 and F1 rats in a two-generation reproduction toxicity study after oral exposure up to 1500 ppm Bronopol for 18 weeks
  - No effect in **mammary gland histopathology (females)** observed in rats in a two-generation reproduction toxicity study after oral exposure up to 1500 ppm Bronopol for 18 weeks, and in dogs in three repeated dose toxicity studies (following 4 and 13 weeks exposure)
  - No effect on **female reproductive organ weights**
    - **Ovary –** No effect of Bronopol on ovary weights observed in five repeated dose toxicity study in rats and dogs (4 and 13 weeks oral exposure) and in rabbits (21 days dermal exposure), in F0 and F1 rats

in two two-generation reproduction toxicity studies and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)
- Statistically significant increase of (rel.) ovary weight at the highest evaluable dose group in one of two repeated dose toxicity studies in rats (following 13 weeks oral exposure). As the ovary weight was within the range expected for rats of this age and strain, not reproducible as it was not observed in the other available study, and without histopathological correlate, this observation was not considered toxicologically relevant.

▪ **Uterus** – No effect of Bronopol on uterus weights (with cervix) in rats and dogs in four repeated dose toxicity studies (13 weeks oral exposure), in F0 and F1 rats and offspring in a two-generation reproduction toxicity study and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)

○ No effect on **female reproductive organ histopathology**
- ▪ **Ovary** – No effect of Bronopol observed in six repeated dose toxicity study in rats and dogs (4 and 13 weeks oral exposure) and in rabbits (21 days dermal exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies, and in rats and mice in two chronic toxicity/carcinogenicity study (104 weeks oral and 80 weeks dermal exposure, respectively)
- ▪ **Uterus** –No effect of Bronopol observed in three repeated dose toxicity study in rats and rabbits (13 weeks oral and 21 days dermal exposure, respectively), in dogs in three repeated dose toxicity studies (following 4 and 13 weeks oral exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies, and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)
- ▪ **Cervix** – No effect of Bronopol observed in F0 and F1 rats in a two-generation reproduction toxicity study and in dogs in two repeated dose toxicity studies (following 4 and 13 weeks oral exposure, respectively)
- ▪ **Oviduct** – No effect of Bronopol observed in rats and dogs in two repeated dose toxicity studies (13 weeks oral exposure), and in F0 and F1 rats and offspring in a two-generation reproduction toxicity study
- ▪ **Vagina** – No effect of Bronopol observed in rats in a repeated dose toxicity study (13 weeks oral exposure), in F0 and F1 rats in a two-generation reproduction toxicity study and in dogs in two repeated dose toxicity studies (following 4 and 13 weeks oral exposure, respectively)

○ No effect on the age at **balanopreputial separation** in F1 offspring in a two-generation reproduction toxicity study

○ No effect on sperm parameters, including **sperm numbers, morphology** or **motility** in F0 and F1 rats in a two-generation reproduction study following exposure to up to 1500 ppm Bronopol for 18 weeks

○ Unspecific effects on **male reproductive organ weights** primarily attributed to significant systemic toxicity
- ▪ **Testis** – No effect of Bronopol on testis weights in rats in three repeated dose toxicity studies in rats and rabbits (13 weeks oral and 21 days dermal exposure, respectively), in dogs in three repeated dose toxicity studies (4 and 13 weeks oral exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies, and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)

- Statistically significant decrease of (abs.) testis weight at the highest evaluable dose group toxicity in one of three repeated dose toxicity study in rats (13 weeks oral exposure) was confounded by reduced body weight. As the decrease of the testis weight was not observed in the other available study, and without histopathological correlate, this observation was not considered toxicologically relevant.

  - **Epididymis** – No effect of Bronopol on epididymis weights observed in rats in two repeated dose toxicity studies (13 weeks oral exposure), in F0 and F1 rats in a two-generation reproduction toxicity study and in dogs in two repeated dose toxicity studies (following 4 and 13 weeks oral exposure)
  - **Prostate** – No effect of Bronopol on prostate weights in rats and dogs in three repeated dose toxicity studies (4 and 13 weeks oral exposure), in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure), and in F0 and F1 rats in a two-generation reproduction toxicity study
  - **Seminal vesicles** – No effect of Bronopol in rats in a repeated dose toxicity study (13 weeks oral exposure), a two-generation reproduction toxicity study and a chronic toxicity/carcinogenicity study (104 weeks oral exposure)

- No effect on **male reproductive organ histopathology**
  - **Testis** – No effect of Bronopol on testis histopathology in four repeated dose toxicity studies in rats and rabbits (13 weeks oral and 21 days dermal exposure, respectively), in dogs in three repeated dose toxicity studies (4 and 13 weeks oral exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies, and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)
  - **Epididymis** – No effect of Bronopol on epididymis histopathology in rats in two repeated dose toxicity studies (13 weeks oral exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies and in dogs in two repeated dose toxicity studies (4 and 13 weeks oral exposure)
  - **Prostate** – No effect of Bronopol on prostate histopathology in rats in a repeated dose toxicity study (13 weeks oral exposure), in F0 and F1 rats in two two-generation reproduction toxicity studies and in dogs in three repeated dose toxicity studies (following 4 and 13 weeks oral exposure)
  - **Seminal vesicles** – No effect of Bronopol on seminal vesicles histopathology in rats in a repeated dose toxicity study (13 weeks oral exposure) and a two-generation reproduction toxicity study
  - **Coagulating gland** – No effect of Bronopol on coagulating gland histopathology in F0 and F1 rats in a two-generation reproduction toxicity study after oral exposure up to 1500 ppm Bronopol for 18 weeks

- No specific effects on most sensitive but not diagnostic parameters related to reproduction/development, including **time to mating, fertility**, **gestation length, fertility, numbers of implantations or corpora lutea, number of ovarian follicles, pre-implantation loss, pup survival index**, **offspring sex ratio, functional observation battery, motor activity.**
  - Reduced **litter size, litter viability** and **number of live births** and increased **post-implantation loss** in F1 rats in a two-generation

reproduction toxicity study, with suggested possible relationship to maternal toxicity; no effect on F0 rats of the same study or in rabbits in a prenatal developmental toxicity study
    - **Dystocia** observed in F0 rats of a two-generation reproduction toxicity study, gross and histopathologic findings did not reveal specific cause, detailed examination suggested possible relationship to maternal toxicity
    - Transient reduced **litter/pup weights** in offspring of F0 rats, at PND 7 and 14 in a two-generation reproduction toxicity study, no significant difference at PNDs 1, 4 or 21 and the partial recovery of body weight by PND 21 correlated with increased water consumption and body weight gain of lactating females, also no effect in offspring of F1 rats observed
    - **Fetal skeletal findings** (including axial skeletal malformations and general skeletal ossification) were observed in rabbits in two prenatal developmental toxicity studies, only in the presence of maternal toxicity and give no indication for a specific effect
    - **Reduced fetal weight** and increased incidence of runted fetuses occurred in rabbits of one pre-natal developmental toxicity study, however the impairment of fetal weight occurred at a systemic toxic dose and is considered secondary to maternal toxicity
- Unspecific effect on **adrenals** and **brain weights** in rats in the absence of histopathological changes, confounded by systemic toxicity (reduced body weight), no effect in other studies in rats and dogs
- Evidence of target organ toxicity found in **kidney** due to significant histopathological changes and impact on kidney weight in rats in several studies, clearly demonstrating systemic toxicity

Table 99: WoE for potential EAS-mediated endocrine activity of Bronopol

- No *in vitro* mechanistic evidence of the potential for **ER activity**
  - No effect in 20 assays after 2 – 80 h (Bronopol showed no effect on receptor binding, protein stabilization, regulation of gene expression, regulation of transcription factor activity or cell proliferation) based on **ToxCast model prediction**
  - No ER bioactivity predicted based on ToxCast(TM) "Endocrine Receptor (ER) ER Model (US EPA)
- No interaction with **ER** predicted for Bronopol based on QSAR model
- No consistent *in vitro* mechanistic evidence of **AR activity**
  - No effect in 12 assays after 8 – 72 h (Bronopol showed no effect on receptor binding, protein stabilization or regulation of transcription factor activity) based on **ToxCast model prediction**
  - Changed regulation of transcription factor activity was only found in 1 of 6 assays, only positive for antagonistic activity above the cytotoxicity limit, indicating no specific direct receptor interaction
- No interaction with **AR** predicted for Bronopol based on QSAR model
- Negative for Androgen receptor transactivation (based on two published OECD TG 458, one conducted before TG was established)
- No *in vitro* mechanistic evidence of an effect on **steroidogenesis**
  - No effect in 20 assays after 1 h and 48 h (Bronopol showed no effect on the capacity to interfere with endogenous steroid hormone biosynthesis and metabolism) based on **ToxCast model prediction**
  - Changed regulation of transcription factor activity only found in 1 of 21 assays, only positive above the cytotoxicity limit, indicating no specific direct activity
- No **steroidogenesis**-related activity predicted for Bronopol based on QSAR model
- *In vivo* no effect on **Phase I enzyme induction** observed in rats of a repeated dose study after 13 weeks of oral exposure to Bronopol up to 1000 ppm

To conclude, the potential EAS-mediated adversity of Bronopol has been assessed in a comprehensive set of toxicity studies, thus the EAS-mediated adversity with regard to humans and mammals (as non-target organisms) should be considered as sufficiently investigated. While these studies identified the kidney as target organ, no evidence for any EAS-mediated adversity has been found for Bronopol.

According to the ED Guidance document, "to have the T-mediated adversity with regard to humans and mammals (as non-target organisms) sufficiently investigated, the thyroid parameters foreseen to be investigated in the following studies OECD TGs 407, 408, 409 (and/or the one-year dog study, if available), 416 (or 443 if available) and 451-3 should have been measured and the results included in the dossier." This includes the blood levels of T3 and/or T4 and TSH, thyroid weight, thyroid histopathology, and HDL/LDL ratio. Among these, the determination of the HDL/LDL ratio is only recommended in OECD TG 408, and is included only in the revised version adopted 25 June 2018. Therefore, the measurement of this parameter was not foreseen at the time the sub-chronic toxicity studies on Bronopol were conducted. Consequently, this parameter is not considered in this database. However, as all the other above-mentioned parameters were addressed in the available studies, the T-mediated adversity with regard to humans and mammals (as non-target organisms) should be considered as sufficiently investigated.

As described above, the organ weights and histopathology, also including the thyroid, have been assessed in several animal studies. In the available two-generation reproductive toxicity studies, most changes in organ weights were sporadic. However, an increase in

absolute and relative thyroid weight in high dose F0 females correlated to histopathological effects in these animals [Carney 2008]. The absolute (0.0191 g) and relative thyroid weight (0.0064 g/100) were within the range of historical control values for absolute (0.0164-0.0192 g) and relative (0.0056-0.0066 g/100) thyroid weights. F0 females given the mid dose of 0.05% and high dose of 0.15% Bronopol had a very slight, treatment-related, overall increase in the numbers of dilated thyroid follicles and many of these dilated follicles were lined by flattened epithelial cells. However, there was no appreciable hyperplasia/hypertrophy or differences in the colloidal staining properties compared to that of the controls. In the absence of a thyroid weight effect in F1 females, thyroid histopathology was not triggered. Similarly, based upon an absence of change in thyroid weight, thyroid histopathology was not triggered in males [Carney 2008]. In the other two-generation toxicity study, an increase in thyroid/parathyroid weight was reported for adult rats only for F1 males and occurred only in the high dose group, while systemic toxicity was already observed in the mid dose animals in both generations [Schardein 1987]. As in this study, an effect on the thyroid was observed in males but not female rats, the occurrence of effects in females in the first-mentioned two-generation reproduction toxicity study does not indicate a higher susceptibility of pregnant rats. In one of the sub-chronic repeated dose toxicity studies, slight but statistically significant increase of (rel.) thyroid weight were observed in males at 80 mg/kg bw/d, which were confounded by severe systemic toxicity. In the other available sub-acute and sub-chronic toxicity studies in rats and dogs and the chronic toxicity/carcinogenicity study in rats, no evidence for any change in thyroid weight or thyroid histopathology has been found upon prolonged exposure to Bronopol. Taken together, although thyroid adversity is quite common in repeated dose-type toxicity studies, especially in the rats, the available studies on Bronopol indicate no such adverse effect on the thyroid organ for this substance.

In one 90-day rat toxicity study with a 4 week recovery group, an increase in T3 was observed in recovery males only. The statistically significantly higher T3 levels in high-dose recovery males were considered due to chance by study authors, since the differences were only slight and individual control values of concurrent control animals ranged all below the 2s limits of historical controls. No change in T4 or TSH was observed in these animals, and T3, T4, and TSH levels were comparable to controls for main group males and females and recovery females at all dose levels. Considering that no change was observed in these hormone levels during treatment with Bronopol, this is interpreted as a sporadic change unrelated to treatment.

Taken together, these CF level 4 and 5 studies do not provide clear evidence of an adverse effect of Bronopol on the thyroid. To further strengthen this conclusion, the lines of evidence for a potential thyroid-disruptive effect are assessed in the following (see table 100 and 101, based on the lines of evidence in section A.6.8).

Table 100: WoE for potential T-related adversity of Bronopol

- No consistent indications of **T-related adversity** of Bronopol as follows:
  - No effects of Bronopol on **thyroid histopathology** observed in rats in three repeated dose toxicity study (following oral exposure for 13 weeks) and a two-generation reproduction toxicity study, in four repeated dose toxicity studies in dogs (following oral exposure for 4 and 13 weeks) and rabbits (following 21 days dermal exposure), and in rats and mice in two chronic toxicity/carcinogenicity studies (104 and 80 weeks oral exposure, respectively)
  - In another two-generation reproduction toxicity study (with exposure duration of 18 weeks), histopathological changes were observed only in F0 females (histopathology of thyroid not performed on F1 females due to absence of change in thyroid weight)

- No effect of Bronopol on **thyroid weights** was observed in rats in one repeated dose toxicity study (following oral exposure for 13 weeks), in four repeated dose toxicity studies in dogs (following oral exposure for 4 and 13 weeks) and rabbits (following 21 days dermal exposure), and in rats in a chronic toxicity/carcinogenicity study (104 weeks oral exposure)
- In a two-generation reproduction toxicity study (with exposure duration of 18 weeks), increased thyroid weights were observed in F0 females at the highest dose level tested (1500 ppm), which was not found in another two-generation study and not in the F1 animals (tested up to 1500 ppm)
- In another two-generation reproduction toxicity increased (rel.) thyroid weights were in adult F1 males of the high dose group (200 mg/kg bw/d), without histopathological correlate and related to the lower mean body weight of these animals
- No effect on **cholesterol level** was observed in rats and dogs in two repeated dose toxicity studies following 13 weeks oral exposure

- No reported treatment-related effect on most sensitive but not diagnostic parameters, including **functional observation battery, motor activity and neurobehavioral changes** investigated in rats and dogs in two repeated dose studies (with 13 weeks exposure duration)

- Evidence of target organ toxicity found in **kidney** due to significant histopathological changes and impact on kidney weight in rats in several studies, clearly demonstrating systemic toxicity

Table 101: WoE for potential T-related endocrine activity of Bronopol

- No *in vitro* agonist activity at **thyroid hormone receptor (THR)**
- Interaction with THR only found in one out of four *in vitro* assays; *in vitro* **antagonist activity at THR** was only observed in the presence of cytotoxicity, indicating no specific direct receptor interaction
- No *in vitro* inhibition of **thyroperoxidase (TPO) enzyme**
- No *in vitro* inhibition of **Sodium-ioidi symporter (NIS)**
- No *in vitro* inhibition of **deiodinases (DIO)**
- No *in vitro* effects on modlatros of TH transport via interaction with TH plasma binding proteins.

- No interaction with **THR** predicted for Bronopol based on QSAR model

- *In vivo* no effect on **thyroid-stimulating hormone level (TSH)** and **T4 level** observed in rats of a repeated dose study after 13 weeks oral exposure to Bronopol up to 1000 ppm; also no effect was observed on **T3 level** in the rats within the main study and recovery females, only slight effect in recovery males was considered sporadic, individual values within the historical controls

As can be seen in table 100 and 101 above, the available information provides no evidence for endocrine activity as well as adversity with regard to the thyroid. Beside the absence of a consistent evidence in the thyroid organ, no changes in behavior or activity as a possible consequence of hyper- or hypothyroidism have been observed in the available studies (see summary in table 100 and table 101).

Estimated residues in food from the intended use were 0.45 mg a.s./kg food (see AR B.3.3.7, p. 535), thereby exceeding the default MRL of 0.01 mg/kg that applies for

bronopol (according to Art 18(1)(b) Reg 396 / 2005).

A.6.9.1.2. Conclusion

To conclude, the EATS-mediated parameters have been sufficiently investigated with regard to humans and mammals as non-target organisms. The available mammalian toxicity studies demonstrate that the principal target organ of Bronopol is the kidney, wherein adversity is not considered to be mediated by an endocrine mode of action. Furthermore, as the available animal studies do not provide consistent evidence for any EATS-related adversity which may be linked to an endocrine activity, the substance Bronopol does not meet the ED criteria with regard to humans and mammals as non-target organisms (scenario 1a of ECHA/EFSA, 2018, section 3.4.4.1).

A.6.9.2. Other non-target organism

The current guidance for biocides states that an assessment of other non-target organisms (NTOs) is required (ECHA/EFSA, 2018). With respect to the terrestrial compartment, the endocrine disrupting potential of Bronopol is addressed by the conducted assessment for human health based on *in silico* models, *in vitro* assays and mammalian data. It was concluded that the ED criteria are not met for Bronopol. There are no additional data available on other taxa (*e.g.* birds) containing information useful for the evaluation of endocrine disrupting properties.

With regard to the aquatic compartment, no sub-lethal adverse effects on development and reproduction were observed in long-term toxicity studies on fish (OECD TG 210, and OECD TG 215) and invertebrates (OECD TG 211). These studies are assigned to Level 4 in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (as included in the OECD GD 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, 2018), meaning they only provide data on adverse effects on endocrine relevant (and not endocrine-specific) endpoints. Furthermore, in the long-term toxicity test on invertebrates the only endpoint which is relevant for the ED assessment (production of male offspring as described in Annex 7 of OECD TG 211) is an optional one, which is not standardly assessed. Although no defined sex-ratio analysis on the parental daphnids was performed in the long-term toxicity studies on invertebrates included in the joint active substance dossier for Bronopol, the absence of any reported visual size differences (male daphnids are smaller than female one) among the adult daphnids after 21 days of exposure supports the conclusion that the test substance had no significant impact on the sex ratio of the daphnids. This is further supported by the fact that no ephippia (dormant eggs) were reported during the test period. The ephippia are commonly created sexually in the presence of male daphnids. The male daphnids are needed to fertilize the haploid eggs. Due to the absence of ephippia, it can be concluded that the female daphnids were neither stressed nor were any male daphnid present at any time during the 21d exposure period.

To confirm the conclusion that was drawn for the ED properties of Bronopol based on the results of the long-term toxicity studies according to OECD TGs 211, 215, and 210 and for further investigations on potential endocrine mechanisms, Level 3 studies are required. To consider the E, A, S and T modalities for NTOs sufficiently investigated, the ECHA/EFSA Guidance recommends the performance of the 'Fish short term reproduction assay' (FSTRA, OECD TG 229) and the 'Amphibian metamorphosis assay' (AMA, OECD TG 231).

On 24/11/2020 (communications [redacted]; [redacted]), the eCA required the following: "Although the document fits the ECHA/EFSA Guidance, there are some points that should be amended: Related to the non-target organisms, the EFSA/ECHA Guidance suggests in the point 4 and table 9, the Level 3 assays that should be conducted to have such NTOs sufficiently investigated, for EAS-modalities and T-modality as well." On 2/03/2021 applicants jointly replied that Bronopol does not produce EATS-mediated adversity in available studies level 1 and 2, and on mammals level 4 and 5. They did not provide any studies for NTOs neither a proposal.

However, with the finalisation of the test guideline for the Xenopus embryonic thyroid

signalling assay (XETA, OECD TG 248) an adequate alternative for the AMA is available. The XETA has been preferred for the ED assessment of Bronopol based on the absence of indication for endocrine activity in humans and in mammals as non-target organisms. Annex A of the ECHA/EFSA Guidance states: if no adversity on thyroid was detected in a complete mammalian data set, a negative XETA would be sufficient to support that T-mediated adversity is unlikely on non-target organisms other than mammals (ECHA/EFSA, 2018). Additionally, and despite that some Modes of Action are not well covered, the XETA is considered adequate, because the available information in US EPA Dashboard and other open literature suggest that these MoAs are unlikely (see LEVEL 2 information in Table 2).

Hence, on 14/06/2021, a XETA is agreed for T-modality, as suggested in the ECHA/EFSA guidance on ED. For EAS modalities an OECD TG 229 FSTRA study is agreed.

Study protocols and range-finding tests were asked for twice by eCA ES with a final deadline of 08/04/2022. They were received, meeting such deadline, when the tests were already on-going.

The eCA found some issues with the top dose concentrations selected derived from the range-finding tests to perform the final studies:

- OECD TG 248: In May 2022, regarding XETA assay, eCA communicated the applicants via R4BP3 "No results of that range-finding test are still provided, and the concentrations that are finally tested are missing, as well as the concentration range. eCA kindly reminds you to take into consideration the recommendations about concentrations tested included in OECD GD 150 "The top dose or concentration should be sufficiently high to give clear systemic (*i.e.* non endocrine-specific) toxicity in order to ensure that a wide range of exposures (high to low) is tested".". Further, several deficiencies were detected in the range-finding tests and the selected concentrations. Some deficiencies were amended, but test concentrations were not changed/repeated by laboratories. Also the eCA recommend the applicants to identify degradation products due to Bronopol rapid degradation, but they refuse to do so as this was not in the scope of these studies.

- FSTRA: such concerns were communicated to the applicants twice in June and July (05/07/2022): for FSTRA "eCA considers that a higher concentration than 1 mg/L should be tested" but applicants replied in 25/07/2022 that the range finding test had been designed in accordance with OECD TG 229, using the spacing factor of 10 and therefore is also consistent with the guidance.

10/03/2022: applicants inform on delays on the FSTRA study.

14/04/2022: applicants inform on delays on the XETA study.

Final deadlines were established by R4BP3 on 23/05/2023 for the submission of the studies: communication number [redacted] with DL 30/06/2023 for OECD TG 248 and communication number [redacted] with DL 01/09/2023 for OECD TG 229.

After such delays in the studies, XETA was submitted by the applicants on 30/06/22 and FSTRA on 23/09/22.The XETA was performed according to the OECD TG 248 and showed conflicting results regarding the observed induction of fluorescence for any of the tested Bronopol concentrations (0.617, 1.85, 5.56 and 16.7 mg/L in water) compared to the respective control, observed induction of fluorescence: *i.e.* a significant effect (> 12 %) on fluorescence in the highest concentration in run 1 in the non-spiked exposure group, a significant reduction (> 12 %) of fluorescence in run 2 and a significant increase (> 12 %) of fluorescence in run 3 - both in the T3-spiked exposure groups. Bronopol is considered as thyroid inactive and T-mediated adversity of Bronopol on NTOs is unlikely [[redacted] 2022].

However, due to deviations from the OECD Guidance the results can be used as supportive information, as suggested by the experts after the e-consultation to the ED EG launched in February 2023.The main concern is due to the low concentrations that might be not high enough to generate effects in the XETA assay. The comments from the ED EG experts were received and the results of such consultation finalised on 15/03/2023. The ED EG

suggested that the XETA assay meets the validity criteria but cannot provide a conclusive answer to the T-modality of bronopol because of the following reasons:

o Test concentrations of this study might not be high enough to induce the response and because the results are negative.

o The individual runs show conflicting results regarding the observed induction of fluorescence.

o The difficulties to interpret the results due to:

- The exact measured concentrations of Bronopol during the exposure period are not well known.
- Degradations products are not identified and measured in this test.

Hence, both the ED EG (only as consultory body) and the eCA considered this test as supporting information.

In addition, the results of the FSTRA (conducted following OECD TG 229), showed that Bronopol (at 0.110, 0.330 and 1.00 mg/L water) did not cause any adverse effects related to the endocrine system and reproductive performance of the tested fish species (fathead minnow, *P. promelas*). Consequently, EAS-mediated adversity of Bronopol on NTOs can be considered unlikely [2022]. Due to deviations from the OECD Guidance the results can be used as supportive information. The main problem is also related to the low concentrations, that as in the XETA assay, might be not high enough to generate effects in the FSTRA assay.

Additional information publicly available from non-guideline studies. *et al.* (2018) exposed three-week old *X. laevis* tadpoles stage 51-52 for three weeks with Bronopol as part of equimass mixtures (0.1, 1, 5 or 10 µg/L) of a total of 23 chemicals (of suspected endocrine activity towards any relevant hormone receptors) to simulate realistic environmental conditions. The 6-week-old tadpoles are still at premetamorphic stages (stage 55–56). More advanced tadpoles that reached stage 56 were discarded to minimize effect of metamorphosis on gene expression. Hence, the exposure took place during the NF 51-55. The results are referred to the premetamorphic tadpoles NF 54-55 exposed. Despite that the mixture coulc be considered environmentally relevant and represent a worst-case approach, it is not considered relevant for the evaluation of Bronopol since the results obtained are related to a mixture of 23 chemicals (including Bronopol). However, since that publication is based on a previous study (*et al.*, 2015 and 2016c). These studies mentioning that Bronopol is not considered to have T-effects, could be used as supportive information.

With regard to fish, no further test guideline conform study assessing endocrine disruptive properties could be identified in open literature. However, Bronopol has been evaluated and approved as active pharmaceutical ingredient in veterinary medicine intended for fish treatment (product name: Pyceze; *e.g.* US Patent Number 6,160,023). More specifically, this Bronopol-based veterinary medicinal product has been approved for the prevention or reduction of fungal infection of farmed Atlantic salmon and rainbow trout, both fish and eggs. Recommended treatment foresees daily exposure of eggs for 30 min from 24h post-fertilization until hatching, and daily treatment of fish for up to 14 consecutive days (30 min/day). Several publicly available studies assessed the efficacy of Bronopol for treatment of fish infected with fungus Saprolegnia species, such as and (2007); some of them also provide information on potential adverse effects of exposure of fish or eggs to Bronopol albeit tested under non-guideline, non-standardized conditions. (2002) found no treatment-related adverse reactions in rainbow trout after exposure to Pyceze for 30 min on 15 consecutive days. *et al.* (2008) found that exposure of rainbow trout or ayu eggs to 50 and 100 ppm of Bronopol from the stage of eye development to hatching did not increase the incidence of deformities.

Based on all available information, no significant endocrine disruptive potential of Bronopol towards non-target organisms is indicated. Neither the literature data, nor the results of

the FSTRA and the XETA suggest any endocrine activity, which is in consistence with the results achieved in *in vitro* assays and *in silico* predictions. Those conclusions are further confirmed by Bronopol's approved usage as active pharmaceutical ingredient in veterinary medicine for the prolonged, repeated treatment of fish or eggs, *i.e.* the very sensitive early life stages of fish.

Therefore, it is concluded that EATS-mediated adversity to aquatic organisms is very unlikely.

As described in detail in the joint active substance dossier, Bronopol has a very short half-life in the environment and rapidly degrades under realistic environmental conditions which involve abiotic as well as biotic degradation pathways (hydrolysis, (primary) biodegradation, photolysis). The substance's environmental relevance is therefore limited to a certain extent.

Considering potential metabolites or degradation products of Bronopol that are generated or released into the environment, three major substances could be of interest with regard to exposure of NTO: 2-nitropropane-1,3-diol (CAS 1794-90-7), 2-bromo-2-nitroethanol (BNE, CAS 5437-60-5) and tris(hydroxymethyl)nitromethane (TNM, CAS 126-11-4), which are environmental degradation pro¬ducts of Bronopol. For 2-nitropropane-1,3-diol and BNE no literature data were available. However, for all three metabolites QSAR analyses (QSAR Toolbox and OASIS Times) indicated no binding to or activation of estrogen receptor, androgen receptor or inhibition of aromatase (data not shown). For TNM this is furthermore supported by *in vitro* data from the EDSP21 screening initiative. The substance showed neither binding nor (trans)activation of androgen receptor (AR; 9 *in vitro* assays available) in sub-cytotoxic concentrations, and no (trans)activation of estrogen receptor (ER; 15 *in vitro* assays available) and thyroid receptor (ThR; 3 *in vitro* assays available). The data/results and concentrations are only reported graphically and in a tabular format; cytotoxicity is reported as cytotoxic limit in the graphical overviews [EDSP21; [redacted] *et al.* 2014; [redacted] *et al.* 2013; data not shown above].

Related to the ions Br^- generated during the metabolization of Bronopol, which are known to be NIS competitive inhibitors, Wang *et al.* 2019 indicated that Bronopol does not show any effect related to the Inhibition of sodium iodide symporter (NIS). Further investigations on the degradation products of Bronopol were not carried out based on the announcement by ECHA in their webinar on 19 June 2018, that degradation products are not subject to the ED assessment. Nevertheless, the metabolites of Bronopol have no endocrine disrupting properties either which is based on a comprehensive literature search ([redacted], "Assessment Report for BNP Metabolite Literature").

A.6.9.2.1. Conclusion

The data are not sufficient to conclude on the ED-mediated adversity of Bronopol in non-mammalian non-target organisms in the environment when strictly applying the ECHA/EFSA ED Guidance (2018) due to the uncertainities on EATS modalities based on the available mechanistic studies in fish and amphibia. However, a Weight of Evidence approach was done based on the whole data package available, not showing any evidence for T-modality effects.

After the ED EG consultation results, the eCA suggested such WoE approach for concluding that Bronopol does not meet T criterion based on all the information available and knowing that the applicants insisted of the reliability (RI = 1) of the submitted assays. All this information was provided to the applicants.

After the accordance check of ECHA and hence the peer review already started, the applicants asked for stopping the peer review from our side, which was commented by ECHA to be not possible anymore. The applicants asked for such possibility in an email from 28/04/2023, quoted from applicants' email: "*From our perspective the current XETA (in accordance with GLP) test according to OECD TG 248 is reliable and valid resulting in a Klimisch score of 1. It met the validity criteria lined out in the respective test guideline. Additionally, the test concentrations used for the main test were chosen based on results*

from pre-tests and standard approaches. In June 2021 eCA and the applicants agreed in pursuing this specific approach to conclude on the T criterion for non-mammalians. Therefore, the applicants cannot understand this statement. Lastly the applicant would like to know which further tests could be required in the opinion of the eCA, bearing in mind that further testing would at least need 14-24 months of additional time. This is due to the order situation on the market and cannot be influenced by the applicants. Our understanding is that, if we do not get the chance to perform the tests, this would be considered by the Commission as a data gap and the concerned product types of Bronopol dossier would not be approved. If that consideration is correct, we highly recommend to withdraw also the other PTs (2,11,12). Please let us take up the points from ECHA and revise the dossier before it goes into the per review process".

During the RCOM, some concerns were raised by the ENV WG members regarding the relevance of the studies provided on ED NTOs. For the BPC ENV WG the applicants provided a position paper insisting again on the reliability RI = 1 for OECD TGs 248 and 229 assays.

Regarding OECD TG 229, FSTRA, the test was considered valid by the eCA (meet validity criteria) but due to low testing concentrations, some uncertainties made it useful just as supporting information as well. The WG agreed with eCA position related to it.

Regarding XETA, several WG members considered that T-modality is not sufficiently investigated, raising their concern on T properties of bromide ion, and showing preference for an AMA test.

A data gap is then identified by the ENVWGIII2023, where it is concluded that further assays are needed to assess the possible Bronopol endocrine disrupting properties.

A.6.9.3. General conclusion

It is possible to conclude that Bronopol is sufficiently investigated, and no adversity based on "EATS-mediated" parameters were found, thus the general conclusion is: Bronopol does not meet ED criteria there is no "EATS-mediated" adversity with regard to humans and mammals as non-target organisms. This is Scenario 1a included in Table 5 of ECHA/EFSA Guidance. However, it cannot be concluded the ED properties for non-target organisms other than mammals, and further information must be requested. This is the Scenario 2a (iii) included in Table 5 of ECHA/EFSA Guidance.

A.6.10. References

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A.6.11. Annexes

A.6.11.1. Annex I: ED criteria for biocides

[redacted]

A.6.11.2. Annex II: QSAR prediction reports

[redacted]

A.6.11.3. Annex III: Documentation of systemic and targeted literature search

[redacted]

A.6.11.4. Appendix E

A.6.11.5. CompTox

A.7 Additional Labelling

Not relevant.

A.8 Assessment of exclusion criteria, substitution criteria and POP

A.8.1. Exclusion criteria

A.8.1.1. Assessment of CMR properties

Criteria (BPR Article 5[1])	Assessment
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, carcinogen category 1A or 1B	Active substance is not classified and does not meet the criteria to be classified as Carc. Cat. 1A or 1B.
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, mutagen category 1A or 1B	Mutagenicity results in mammalian cells are equivocal and *in vivo* mutagenicity cannot be completely disregarded. Therefore, no conclusion can be drawn. According to the BPR, a gene mutation study in bacteria, a chromosomal aberration study and a gene mutation study in mammalian cells are required to study genotoxicity *in vitro*. If any of these studies give a positive result, a corresponding follow-up with an appropriate *in vivo* study should be performed. The most recently conducted mammalian cell gene mutation study was weak positive. This result was followed-up *in vivo* with a UDS assay, which when the eCA started the evaluation (03/2022), was still included in the BPR guidance for information requirements (Part A). The validity and sensitivity of the UDS test was scientifically questioned. This is in line with the new versions of the BPR application guidance Part A for HH and BPR (29/03/2022 and 15/04/2022, respectively), the UDS assay was removed. The guidance came into force on 29/09/2022, and the eCA submitted its finalized dCAR to the Applicant for its 30-day commenting period on 14/02/2023. During the trilateral discussions prior to the Working Group (23 June 2023), the eCA strongly advised the Applicant about a possibility to conduct the corresponding study to study *in vivo* mutagenicity (comet or TGR) noting that the acceptability of the UDS was questioned in the RCOM by the CAs. The Applicant decided to follow a WoE approach, as the timeframe did not allow the applicant to perform the appropriate study, which was not accepted by the WG. A data gap for a reliable *in vitro* gene mutation test in mammalian cells was identified by the Human Health WG. Given the too short timeframe of 10 days following the WG to perform the study, no request for a new

	study was made by the WG.
Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, toxic for reproduction category 1A or 1B	Active substance is not classified and does not meet the criteria to be classified as Repr. Cat. 1A or 1B.
Conclusion on CMR properties:	**Bronopol does not fulfil criteria (a) and (c) of Article 5(1). Due to a data gap on genotoxicity no conclusion can be drawn respecting to criterion (b).**

A.8.1.2. Assessment of endocrine disrupting properties

Criteria (BPR Article 5)	Assessment
Active substances which, on the basis of the criteria specified pursuant to the first subparagraph of paragraph 3 are considered as having endocrine-disrupting properties that may cause adverse effects in humans and to the environment.	The assessment should be made in accordance with the scientific criteria set out in Commission Delegated Regulation (EU) 2017/2100.
Conclusion on ED properties:	**Bronopol does not fulfil criterion (d) of Article 5(1) for human health. However, no conclusion can be drawn for non-target organisms.**

A.8.1.3. PBT Assessment (following Annex XIII to Regulation (EC) No 1907/2006)

Assessment of persistence

Screening

In this context it is important to note that a substance may consist of more than one constituent or that it may form transformation or degradation products. If the substance contains one or more constituents with PBT/vPvB properties in individual amounts ≥ 0.1% (w/w) or if transformation/degradation products with the respective properties in individual amounts ≥ 0.1% are being generated, the substance must be treated like a PBT/vPvB.

Assessment

Bronopol is not considered persistent due to the following reasons:

- Hydrolysis: rapid hydrolysis is shown at environmentally relevant pH both from hydrolysis studies and literature (abiotic degradation processes seem to be predominant under environmental relevant pH values).
- Ready biodegradability: In one of the studies considerable mineralisation of Bronopol (up to 57%) was observed within the 28-day test period, very close to the target of 60% mineralisation. In a second study, 67-89% mineralisation was achieved after a period of 29 days but failing the 10-day-window (45-55%). In the third reliable test, only a 20% after the exposure period of 28 days was observed. There is a contradiction in these tests, with different results among them, the criteria for readily biodegradability is jut fulfilled in one of them but failing the 10-d window.
- There is a supportive study by [redacted] *et al.*, 2010, hydrolysis at 20 ºC and photolysis at 34 ºC, showing that degradation half life of Bronopol in natural waters would be in the range of 0.24 h at 12 ºC:

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

Water sample	BNP				BNE*			
	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

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

- Bronopol rapidly disappears in aquatic media in ecotox studies. For further information on that, please see section A.4.2. The measured concentrations in one of the tests with freshwater algae is included as an example:

Measured Concentrations of Bronopol in Freshwater Algal Medium Samples

Nominal Test Concentration (µg/L)	Sample ID (103A-119A-)	Sampling Time (Day)	Measured Concentration (µg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	<LOQ (2.67)	--
0.0	8	4	<LOQ (2.67)	--
5.5	2	0	5.22	94.9
	9	4	<LOQ (2.67)	--
12	3	0	12.0	100
	10	4	<LOQ (4.00)	--
27	4	0	27.5	102
	11	4	<LOQ (4.00)	--
61	5	0	63.0	103
	12	4	<LOQ (8.00)	--
135	6	0	102	75.4
	13	4	< LOQ (16.0)	--
300	7	0	305	102
	14	4	<LOQ (40.0)	--

- There is monitoring data available in section A.4.1.4 (original study included with the additional documents to the CAR) which shows that no Bronopol has been detected remaining in any of the environmental compartments tested (water, sludge, air…).
- The study OCDE 314 with Bronopol shows a very rapid degradation, both abiotic and biotically.

Considering Bronopol a borderline case for ready biodegradability and the evidence that Bronopol has a fast primary degradation, together with the monitoring data and the rapid disappearance of the a.s. both in the STP simulation and the ecotox studies, it can be considered as not persistent.

Hence, running an additional study on biodegradation would provide little additional insight into the environmental fate of Bronopol beyond what is already available.

The main metabolites are not considered persistent due to the following reasons:

2-BNE

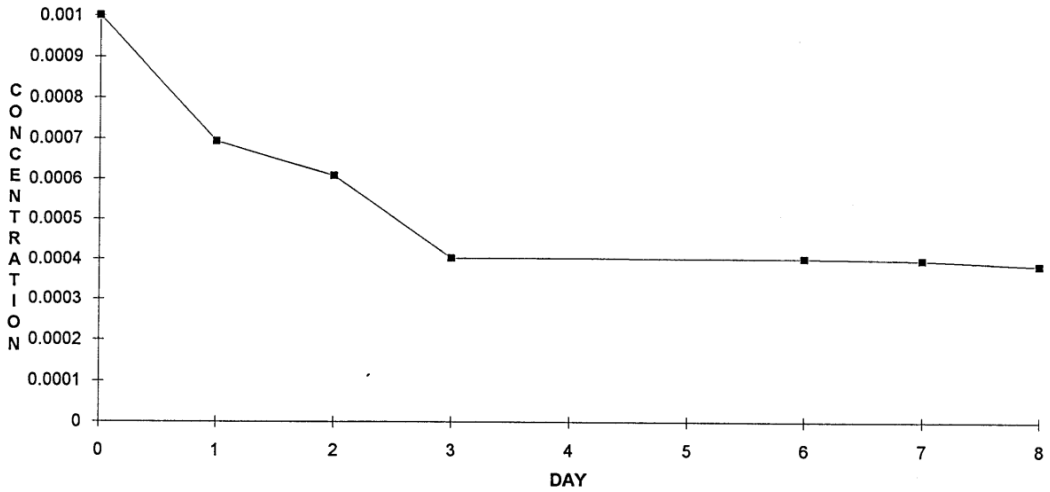
- 2-BNE is readily biodegradable based on a study considered as supporting information.
- There is a valid OCDE 314 study showing the very rapid biodegradation of 2-BNE which clearly indicates that it is not persistent.
- This is also supported by the [redacted] literature data where the degradation of 2-BNE in natural waters is in the range of 8-19 h for a half-life.
- 2-BNE disappears quite rapidly in some of the ecotoxicological tests. These are the measurements in the study with freshwater algae:

Table 3. **Concentrations of 2-bromo-2-nitroethanol measured in the exposure solutions during the 72-hour exposure of *Pseudokirchneriella subcapitata*.**

Nominal Concentration (mg a.i./L)	Measured Concentration (mg a.i./L)[a]			Time-Weighted Average[ab]	Percent of Nominal[a]
	0 Hour	24 Hours	72 Hour		
Control	<0.0027[c]	<0.0028	<0.0024		
0.0098	0.011	0.0073	<0.0024[d]	0.0052	53
0.025	0.027	0.020	<0.0024[d]	0.012	48
0.061	0.067	0.049/0.060[e]	0.0079/0.042[e]	0.034	56
0.15	0.18	0.14	0.054	0.11	76
0.38	0.46	0.35	0.18	0.30	80
0.96	1.0	1.1	0.55	0.89	93
QC[f]#1 0.00499	0.00566 (113)	0.00545 (109)	0.00555 (111)		
QC#2 0.101	0.102 (101)	0.100 (99.5)	0.110 (109)		
QC#3 1.01	1.06 (106)	1.10 (110)	1.08 (108)		

TNM

- The characterisation of 2-Hydroxymethyl-2-nitro-1,3-propanediol (trade name tris-(hydroxymethyl)-nitromethane) (TNM) as major degradation product was included in the study by [redacted] (2002a) for Bronopol, and in the last sampling (28 days), its concentration had fallen below 50% of the peak concentration.
- The hydrolysis data for TNM (literature data) shows a DT50 = 3.4 d at 25 ºC ([redacted] H., 1993). The information is provided in table A-43. TNM is expected to be decomposed to formaldehyde as the only relevant metabolite. As stated in section A.4.1.1, the presence of formaldehyde in a closed system was shown to stabilize TNM. This was expected based on the fact that TNM is synthesized by a reversible reaction of three moles of formaldehyde with one mole of nitromethane. This is clearly shown in the study from [redacted], where a biphasic degradation is taken place:



This means that further degradation of TNM would be expected in the environment as an open system where formaldehyde is not accumulating. Being formaldehyde the only relevant degradation product, which is limiting the decomposition of TNM in the closed system, the degradation is likely to continue in the environment.

- In the readily biodegradation study with TNM, criteria for readily biodegradation are not met, but QSAR data for TNM indicates it is readily biodegradable:

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9679
Biowin2 (Non-Linear Model) : 0.9236
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.1330 (weeks)
Biowin4 (Primary Survey Model) : 3.8646 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.9618
Biowin6 (MITI Non-Linear Model): 0.9460
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.9781
Ready Biodegradability Prediction: YES

According to Guidance on information requirements, Chapter R.11: PBT/vPvB assessment, when Biowin 2 (non-linear model prediction) has a probability >0.5 (in this case is 0.9679) and Biowin 3 (ultimate biodegradation time) is above 2.75 (in this case 3.133), is not the case for being potentially P or vP. Same applies for Biowin 6 (MITI non-linear model prediction) being above 0.5 together with Biowin 3.

- There is further QSAR simulation by CATALOGIC 301C model for predicting biodegradability of chemicals, provided as separate documents, which estimates an ultimate half-life of 1 month, which also supports that TNM is not persistent.
- TNM did not meet the criteria for readily biodegradability but fulfilled the criteria for primary inherently biodegradable based on DOC ≥ 40%. The evidence of slight inhibition of microbial activity could be an explanation for the failure of reaching the "readily biodegradable" threshold.
- The OECD TG 301F study was intended to identify the complete mineralisation of TNM to CO2, no intermediates/metabolites were identified. Therefore, the process of hydrolysis is hard to detect at that study type, even if an abiotic or inhibited control is included in the test. The inhibited controls in the present study showed no cumulative consumption of O2 or production of CO2 over the 28-day period confirming that no mineralisation had occurred due to the absence of any microorganisms that could metabolise TNM. However, this does not confirm the absence of any further degradation processes like hydrolysis. The inhibited control may only detect degradation processes which result in a consumption of oxygen or a formation of CO2 which is not generally the case for hydrolysis. Thus, as hydrolysis is a chemical process, it can only be measured by using substance-specific analytical methods. However, analytical monitoring is not mandatory in biodegradation screening tests and with the intention of investigating the biodegradation behaviour of TNM it was not considered relevant for the OECD TG 301F study.

The hydrolysis data from [redacted], 1993, together with the study [redacted] 2002a and the justification from the OECD TG 301F, and the QSAR data (biowin shows no potential to be P/vP and even predicts ready biodegradability) make it very unlikely this metabolite to be persistent. Although not to be used for persistency assessment, just as supporting information, the degradation of TNM in the STP results on a DT50 = 1.17 days at 20 ºC.

Just for further information:

- According to the US EPA, 2-(hydroxymethyl)-2-nitro-1,3-propanediol was first registered in the U.S. in 1955, as an industrial bactericide and slimicide. Several pesticide products are registered which contain this active ingredient[11]. It is used in the US and included in the list of chemicals manufactured (including imported) or processed in the U.S. for uses under TSCA (Toxic Substances Control Act (15 U.S.C. 2607(a)).[12]

[11] https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-083902_1-Sep-93.pdf
[12] https://cdxapps.epa.gov/oms-substance-registry-services/substance-details/33803

- TNM is also included in the Domestic Substances List Categorization Results of Canada, being categorised as not persistent[13], based on QSAR data, although the criteria are different in its regulation (to be considered persistent, >= 6 month in water and soil, and >= 1 year in sediment).

P Criteria	Assessment
T1/2 > 60 days in seawater, or	Not the case for Bronopol neither the relevant degradation products
T1/2 > 40 days in fresh- or estuarine water, or	Not the case for Bronopol neither the relevant degradation products
T1/2 > 180 days in seawater sediment, or	Not the case for Bronopol neither the relevant degradation products
T1/2 > 120 days in freshwater- or estuarine sediment, or	Not the case for Bronopol neither the relevant degradation products
T1/2 <= 120 days in soil.	There is no experimental data for Bronopol, but due to its low lipophilicity and a high water solubility, it will remain in the pore water rather than moving into the soil.

vP Criteria	Assessment
T1/2 > 60 days in sea-, fresh- or estuarine water, or	Not the case for Bronopol neither the relevant degradation products
T1/2 > 180 days in seawater-, freshwater- or estuarine sediment, or	Not the case for Bronopol neither the relevant degradation products
T1/2 > 180 days in soil.	Not the case for Bronopol neither the relevant degradation products

Conclusion on P / vP properties:	**Bronopol has neither P nor vP properties, neither the relevant degradation products.**

Assessment of bioaccumulation

Screening

With a very low Octanol-water partitioning coefficient experimentally log Kow = -0.42 and estimated by (Q)SAR model log Kow = -0.64, the Bioaccumulation Estimates from Log Kow (BCFWIN v2.17) gives a Log BCF from regression-based method = 0.500 (BCF = 3.162).

The BCF is far below the B criteria.

Regarding the two main degradation products, both 2-BNE and TNM bioaccumulation potential has been estaimated by QSAR with similar results as the parent, very low kow:

2-BNE: Log Kow used: -0.74 (KowWin est);

TNM: Log Kow used: -1.66 (KowWin est).

Assessment

B Criteria	Assessment
BCF > 2000	Not the case for Bronopol neither relevant degradation products

vB Criteria	Assessment
BCF > 5000	Not the case for Bronopol neither relevant degradation products

[13] https://pollution-waste.canada.ca/substances-search/Substance/DisplaySubstanceDetails?Id=126-11-4

Assessment of toxicity

Screening

For information on the short-term aquatic toxicity see Section **¡Error! No se encuentra el origen de la referencia.**.

Assessment

T criterion is met when we use geometric mean concentrations as decided in the ENV WG-IV-2022 due to the rapid degradation of the active susbtance in the ecotoxicological studies. As Bronopol degrades quite rapidly, the geomeans are conservative values. Hence, the EC10 for algae has been recalculated to a geomean value of 0.0048, as EC10 < 0.01 mg/L for algae, Bronopol would meet the T criterion (being the endopoint a conservative value).

The main intermediate product 2-BNE is of similar toxicity than the parent.

The EC50 for 2-BNE to algae is 0.109 mg/L and the EC10 is 0.019 mg/L. Such endpoint does not meet T criteria, it is derived for *P.Subcapitata*, comparable to Bronopol EC10 = 0.021 mg/L (geometric mean measured) for such species. We can state that they are both of similar toxicity, but there is no EC10/NOEC available for 2-BNE referring to the most senstivie species to Bronopol (*Scenedesmus*). Hence, 2-BNE can only be stated to be potentially T.

The metabolite TNM is not toxic.

T Criteria	Assessment
NOEC/EC10 (long-term) < 0.01 mg/L for freshwater or seawater organisms, or	Bronopol, with an EC10 < 0.01 mg/L for algae, meets the T criterion
substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to the CLP Regulation, or	Not the case for Bronopol
there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to the CLP Regulation.	Not the case for Bronopol
Conclusion on T properties:	Bronopol is toxic for algae, hence meets the T property

Summary and overall conclusions on PBT or vPvB properties

Overall conclusion:

Based on the assessment described in the subsections above the submission substance is not a PBT / vPvB substance.

A.8.2. Substitution criteria

Substitution criteria (BPR, Article 10)	Assessment
One of the exclusion criteria listed in Article 5(1) is met but AS may be approved in accordance with Article 5(2)	None of the exclusion criteria is met
The criteria to be classified, in accordance with Regulation (EC) No 1272/2008, as a respiratory sensitiser are met	No
The acceptable daily intake, acute reference dose or acceptable operator exposure level, as appropriate, is significantly lower than those of	No concerns identified

the majority of approved active substances for the same product-type and use scenario	
Two of the criteria for being PBT in accordance with Annex XIII to Regulation (EC) No 1907/2006 are met	No
There are reasons for concern linked to the nature of the critical effects which, in combination with the use patterns, amount to use that could still cause concern, such as high potential of risk to groundwater, even with very restrictive risk management measures	No
The AS contains a significant proportion of non-active isomers or impurities.	No
Conclusion on substitution criteria:	**The substitution criteria in BPR Article 10(1)a-f are not met.**

A.8.3. Assessment of long-range environmental transportation and impact on environmental compartments

	Assessment
The active substance or a degradation product is a persistent organic pollutant (POP) listed in Annex I of ~~EC 850/2004~~ (replaced by Regulation (EU) 2019/1021)	Neither the active substance nor any of the identified degradation products is listed in Annex I of Regulation (EU) 2019/1021.
Assessment of long-range transport potential (LRTAP): Vapour pressure < 1000 Pa and half-life in air > 2 days or Monitoring data in remote area showing that the substance is found in remote regions or Result of multimedia modelling	Vapour pressure: 5.1*10-3 Pa Half-life in air: 20-21 days (for 12 hrs sunlight) ⇨ Conditions fulfilled No monitoring or modelling data available.
The active substance or a degradation product is vP/vB or T?	Neither the active substance nor any of the identified degradation products is vP/vB. The active substance and one of the identifies degradation products (TNM) are not T. The degradation product BNE is potentially T. Based on the available data (only acute toxicity data) a final classification is not possible. According to IR&CSA Guidance Chapter R.11 **¡Error! Marcador no definido.**, the T criterion cannot be decided upon the basis of acute studies alone.
Conclusion on LRTAP/POP assessment:	**Based on the physicochemical properties, Bronopol has a LRTAP, but is not listed as POP in Annex I of Regulation (EU) 2019/1021.**

B. Exposure assessment and effects of the active substance in the biocidal product(s)

B.1 General product information

Table 102: Identification of the product

Name(s) of the product	
Trade name(s) or proposed Trade name(s)	1) [redacted] 2) [redacted] 3) [redacted]
Manufacturer's development code and number of the product	Not applicable
Formulation type	Water soluble powder (SP)

Bronopol is a crystalline solid, whose crystals have a large particle size, are hard wearing, do not disintegrate over time and is readily soluble in water.

Table 103: Complete qualitative and quantitative composition of the biocidal product

Active substance					
ISO or Trivial name	**IUPAC name or other accepted chemical name**	**EC number**	**CAS number**	**Composition / all constituents (upper and lower concentration limit in % (w/w))**	**Concentration in the product in % (w/w)**
Bronopol	2-bromo-2-nitro-1,3-propanediol	200-143-0	52-51-7	-	*100%*

Other components / ingredients of the product					
ISO or Trivial name	**IUPAC name or other accepted chemical name**	**EC number**	**CAS number**	**Concentration in in the product in % (w/w)**	**Function**
n/a	n/a	n/a	n/a	n/a	n/a

The detailed composition is an industrial and commercial secret. Therefore, this information should be treated confidential. Please refer to the Confidential Section of this dossier (Annex VI).

Table 104: Physical, chemical and technical properties

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Physical state at 20 °C and 101.3 kPa	Crystalline solid	Visual determination	GLP	[redacted] 2000 (A3.01.1_02)
Colour at 20 °C and 101.3 kPa	White to yellowish	Visual determination	GLP	[redacted] 2000 (A3.01.1_02)
Odour at 20 °C and 101.3 kPa	Not stated	Statement	non-GLP	[redacted] 2006 (A3.03.3_01)
Acidity / alkalinity	pH = 7.3 at 25 °C (25 % aqueous solution) Acidity/alkalinity test is not required because the pH of Bronopol is neither <4 nor >10.	Internal standard operating procedure (Chemistry Equipment S.O.P.2)	non-GLP	[redacted] 1996 (B3.05_01)
	pH = 5.7 (1 % aqueous solution) Acidity/alkalinity test is not required because the pH of Bronopol is neither <4 nor >10.	analogous to DIN ISO 976	GLP	[redacted] 2000 (A3.01.1_02)
Relative density	1.894 g/mL at 20 °C	92/69/EEC A.3 (displacement method with pycnometer)	GLP	[redacted] 2001 (A3.1.1/01)
	relative density: d20/4 = 1.905 +/- 0.001	OECD TG 109	[redacted] (batch identification [redacted]): 2-Bromo-2-Nitro-1,3-Propanediol, purity 99.7 g/100 g GLP	[redacted] 2001 (A3.01.3_01)
	bulk density: 1100 kg/m^3	In house method (Company statement)	[redacted], purity min. 99.0 % bronopol	[redacted] (2007b) BPD ID B3.06_02

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Storage stability, stability and shelf-life				
Accelerated storage				

	Two samples (A and B) were tested with the following initial Bronopol content: Sample A = 100% Bronopol Sample B = 99.6% Bronopol **Storage conditions** / **Content Bronopol [%]** **12 weeks At 20-25°C** / **Sample A** / **Sample B** (a) at normal RH: 99.8 / 99.5 (b) at 90% RH: 100.1 / 99.6 At 37°C: 100.2 / 99.5 At 45°C: 99.6 / 99.6 In north window: 99.8 / 99.6 **52 weeks At 20-55°C** (a) at normal RH: 100.2 / 99.5 (b) at 90% RH: 99.2 / 99.5 At 37°C: 100.3 / 99.5 At 45°C: 100.3 / 99.5 In north window: 99.3 / 99.4	in-house method	Purity: 99.6 - 100.0% A and B refer to two independent measurements on two different samples. The effect of light on the bronopol content was investigated, the information "in north window" refers to conditions with lower light intensity as north windows are usually exposed to less light than *e.g.* south windows. *According to the Guidance on BPR Vol I*, section 3.6.4.1.1.1, page 68; Manual *on development and use of FAO and WHO specifications for pesticides***,** November 2010, section 4.6.2, page 64, the accelerated storage stability test should be conducted according to the CIPAC method MT 46.3 (storage at 54 ± 2 ºC for 14 days as the default test conditions). However, CIPAC Method MT 46.3 and the FAO/WHO Manual indicate other five temperature/time regimes as alternative test conditions. Alternative conditions are: 4 weeks at 50 ± 2 ºC, 6 weeks at 45 ± 2 ºC; 8 weeks at 40 ± 2 ºC, 12 weeks at 35 ± 2 ºC or 18 weeks at 30 ± 2 ºC. The specified temperatures 37°C and 45°C belong to Option (b), *i.e.* they were tested at 90% RH, whereas at normal RH the complete temperature range given in the header was tested (20-25°C for 12 weeks, and 20-55°C for 52 weeks). The study of [redacted] (1985) shows that bronopol is stable up to 52 weeks at 20-55°C. These tested conditions therefore exceed the test conditions of CIPAC method MT 46.3. Thus, the study gives further information as it 'overfulfills' the conditions described in/needed for CIPAC	[redacted] 1985 (B3.07_03)

<table>
<tr><th>Property</th><th>Result</th><th>Test method applied or description in case of deviation</th><th>Remarks / Discussion / Justification for waiving</th><th>References</th></tr>
<tr><td></td><td></td><td></td><td>method MT 46.3</td><td></td></tr>
<tr><td colspan="5">Long term storage at ambient temperature</td></tr>
<tr><td></td><td>Sample/Temp
<table><tr><td>A@ RT</td><td>99.47 % (0m)</td><td>99.88 % (3m)</td><td>98.83 % (6m)</td><td>98.98 % (12m)</td></tr><tr><td>B@ RT</td><td>98.55 % (0m)</td><td>99.86 % (3m)</td><td>98.3 2% (6m)</td><td>100.12 % (12m)</td></tr><tr><td>A@ 40 °C</td><td>99.47 % (0m)</td><td>99.81 % (3m)</td><td>98.7 5% (6m)</td><td>12m not tested</td></tr><tr><td>B@ 40 °C</td><td>98.55 % (0m)</td><td>100.0 2% (3m)</td><td>99.5 5% (6m)</td><td>12m not tested</td></tr></table></td><td>Oxford Analytical in-house method, GLP</td><td>The study of [redacted] (2004) shows the storage stability of bronopol (2 samples A and B) at ambient storage (RT= real testing) for one year and stored at elevated temperature (40±2 ºC) for 3 months and 6 months. The product can be considered stable over the period tested at ambient storage.

The samples were packaged in white, 1000 mL, screw capped, plastic bottles.</td><td>[redacted] 2004 (B3.07_01)</td></tr>
<tr><td></td><td>Storage time/Temp
(results in table in next row)</td><td>[redacted] internal standard method
[redacted]</td><td>Non-GLP
[redacted]

Batch: [redacted]
purity 99.4 g/100 g

Batch: [redacted]
purity 100 g/100 g

Batch: [redacted]
purity 99.9 g/100 g</td><td>[redacted] 2004 (B3.07_02)</td></tr>
</table>

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References

Long-term stability storage results:

	Bronopol content % w/w		
	Batch [redacted]	Batch [redacted]	Batch [redacted]
Initial	99.4	100.0	99.9
3m@25ºC/60%RH	99.7	99.7	99.9
6m@25ºC/60%RH	100.2	99.9	99.8
9m@25ºC/60%RH	99.7	99.7	99.4
12m@25ºC/60%RH	99.8	99.8	99.7
18m@25ºC/60%RH	99.9	100.0	99.7
24m@25ºC/60%RH	99.7	99.7	99.8
36m@25ºC/60%RH	99.9	99.4	99.9
48m@25ºC/60%RH	99.9	99.9	100.0
60m@25ºC/60%RH	99.7	99.8	99.9
3m@40ºC/75%RH	99.7	99.8	99.6
6m@40ºC/75%RH	100.0	99.8	99.8

This table shows the results of a long-term stability study conducted on 3 batches of bronopol. Samples were examined at 25 ºC/ 60% RH. Testing intervals are three months for the first year and then every six to twelve months thereafter. The samples were stored in two sealed polythene bag liners with a dessicant sachet in the inner bag. The sample were then held in a cardboard box. The appearance of the batches remains the same throughout the trial; pH of the batches was consistent and the content of bronopol does not change significantly over the storage. The results of the stability test illustrate that the product is very stable over the period tested.

In addition, samples were examined at 40 ºC/ 75% RH (accelerated storage stability test) for 3 months and 6 months.

The stability of bronopol is supported in long terms storage stability tests: i) storage stability at ambient storage for one year ([redacted], 2004: see above); ii) storage stability at ambient storage ([redacted]). [redacted] examined different batches of bronopol at 25 ºC/60% RH over the period tested (60 months). Bronopol content during this period does not change significantly over the storage as reported in the table, but the shelf life should be limited at 2 years. After this period (24 months), there was not sufficient sample to test *e.g.* appearance.

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Low temperature stability (liquids)			The physical state of the biocidal product is solid. Therefore, this test is not justified.	
Effects on content of the active substance				

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Light	see above: [redacted] 1985 (B3.07_03)		In this study, the influence of light in the following conditions was addressed: "in north window" refers to conditions with lower light intensity as north windows are usually exposed to less light than *e.g.* south windows. The influence of light was studied in bronopol solid (white or almost white cristallyne powder). In addition, according to the information given for UV/VIS absorption (CAR, Appendix I, Chapter I, p. 519), Bronopol as active substance is stable under light. The substance does not absorb light in the visible range (>290 nm). The current reference in the CAR (to the study of [redacted], 1985) is appropriate for providing information on the light stability since this parameter was indeed tested in the accelerated storage test by [redacted], 1985 (see results above, "in north window"). In this study stability under light was confirmed. Nevertheless, it is marketed in opaque packaging anyway. Photolysis is therefore not expected. On the other hand, it was studied the stability of bronopol in aqueous solutions: when exposed to light over longer periods of time, especially in alkaline conditions, solutions of bronopol may become yellow or brown. There is, however, no evidence of close correlation between colour change and loss of antibacterial activity.	[redacted] 1985 (B3.07_03)

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Temperature and humidity	see above: [redacted] 2004 (B3.07_02)			[redacted] 2004 (B3.07_02)
Reactivity towards container material	Crystalline Bronopol in the dry state is not corrosive per se to metals and other packing materials. Based on experience in use bronopol is not expected to react towards container material. 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. 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.	Experience in use, non-GLP		[redacted] 2000 (A3.17_01)
Technical characteristics				

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Wettability			The representative biocidal products are dry solids and readily soluble in water (water solubility in the range of 300 g/L). Based on a long-time experience in use and handling it is known that bronopol and the representative biocidal products are instantly wettable after pouring in or coming into contact with water. Furthermore, the products are not dispersed in water, but are either homogeneously dissolved in the matrix to be protected or have to be applied as a pre-mix solution. Also, the chemical structure of bronopol, which functional groups include 2 x –OH and a nitro group (–N+O2–) capable of forming hydrogen bonds are, together with the high water solubility, are indicative for a readily wettable behaviour of the substance. Therefore, tests on wettability have not been carried out at active substance approval stage.	waiver

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Suspensibility, spontaneity and dispersion stability			According to the "Manual on the development and use of FAO and WHO specifications for chemical pesticides" (second edition), suspensibility aims to ensure that the active ingredient remains homogeneously dispersed in the spray liquid to give a satisfactory and effective mixture during spraying, but is not applicable to formulation type SP. The products are dry solids and readily soluble in water (water solubility in the range of 300 g/L). No applications which would require or lead to the formation of a suspension or dispersion are foreseen for the biocidal products. The products are not dispersed in water, but are either homogeneously dissolved in the matrix to be protected or have to be dissolved (true solution) in an appropriate solvent (water) prior addition. Therefore, tests on suspensibility, spontaneity and dispersion stability have not been carried out at active substance approval stage.	waiver
Wet sieve analysis and dry sieve test			The products are dry solids and readily soluble in water, therefore tests on wet sieve analysis have not been carried out. According to the ECHA Guidance on the Biocidal Products Regulation (Volume I: Identity/physico-chemical properties/analytical methodology – Part A: Information Requirements, version 2.1, March 2022), the dry sieve test is applicable for dusts and granular formulations. Testing was not performed since the products are no dusts or granular formulations.	

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Emulsifiability, re-emulsifiability and emulsion stability			Test about emulsifiability has not been carried out because the products are not used as emulsions.	waiver
Disintegration time	not applicable		According to the ECHA Guidance on the Biocidal Products Regulation (Volume I: Identity/physico-chemical properties/analytical methodology – Part A: Information Requirements, version 2.1, March 2022), the disintegration time is applicable to all products that are tablets and depend on disintegration of the tablet in a solvent (water) for optimal efficacy. As the products are not in tablet form, this endpoint can be waived.	waiver
Particle size distribution, content of dust / fines	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)
	Particle size distributions: D50 = 683–830 µm D10 = 285–393 µm D90 = 1352–1470 µm	GMP, Method not specified		[redacted] 2007 (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] "[redacted]": 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)
Attrition, friability	The attrition resistance of [redacted] is 99.4 %.	CIPAC MT 178, purity 99.6%, GLP		[redacted] 2020b

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Persistent foaming	not applicable	not applicable	Based on the composition of the representative products (*e.g.* [redacted], [redacted]; [redacted]) which contain ≥ 99% active substance (bronopol), and thus, the absence of surface active components, proteins, blowing agents or other compounds capable of producing foam, persistent foaming properties can be excluded. Also, an application in spray tanks is not foreseen for the biocidal products. Based on a long-time experience in use and handling of the active substance/representative products, it is known that the active substance/representative products do not produce significant amounts of foam if dissolved in water regardless of the a.s. concentration. Therefore, tests on persistent foaming have not been carried out at active substance approval stage.	waiver
Flowability, pourability, dustability	not applicable	not applicable	The products are not preparations, but free-flowing crystalline solids. Therefore, testing is regarded not necessary and this endpoint is waived accordingly.	waiver
Burning rate – smoke generators	n/a			
Burning completeness – smoke generators	n/a			
Composition of smoke – smoke generators	n/a			

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Spraying pattern - aerosols	n/a			
Other technical characteristics	n/a			
Physical and chemical compatibility with other products including other biocidal products with which its uses is to be authorised				
Physical compatibility	No physical incompatibilities are known or expected			
Chemical compatibility	Bronopol (min 99%) is compatible with a wide range of materials including cationic, non-ionic, anionic and amphoteric surfactants; solvents *e.g.* Monopropylene glycol & Dipropylene Glycol Monomethyl Ether; sunscreens, natural extracts, retention aids and flocculants. There are no known incompatibilities with dispersants or rheology modifiers and can be used in formulations such as surface coatings, polymer dispersions, adhesives, printing inks and pigments suspensions. Bronopol is also compatible with many biocides including Quaternary Ammonium Compounds (QAC), isothiazolinones (MIT/CMIT), Methylene bisthiocyanate (MBT) and Dibromonitrilopropionamide (DBNPA).			[redacted] 2007d (B3.09_01)
Degree of dissolution and dilution stability			Not required as the product is not used in a water soluble bag or tablet.	waiver
Surface tension	Not surface active result: 72.70 mN/m temperature: 19.9°C concentration of test solution: 1.0 g/L	92/69/EEC A.5 (OECD harmonized ring method)	GLP	[redacted] 2000b

Property	Result	Test method applied or description in case of deviation	Remarks / Discussion / Justification for waiving	References
Viscosity	n/a, not a liquid			

Table 105: Hazard identification for physical and chemical properties

Hazard class / characteristics	Guideline and Method	Purity of the test substance (% (w/w)	Parameter(s)	Results / Waiver	Reference
Explosives	92/69/EEC A.14, GLP	Min. 99.0%		Not explosive	[redacted] 2015 (no BPD-ID); [redacted] 2007 (A3.10_01)
Flammable gases	--	--	--	n/a, not a liquid nor gas	--
Flammable aerosols	--	--	--	n/a, not a liquid nor gas	--
Oxidising gases	--	--	--	n/a, not a liquid nor gas	--
Gases under pressure	--	--	--	n/a, not a liquid nor gas	--
Flammable liquids	--	--	--	n/a, not a liquid nor gas	--
Flammable solids	92/69/EEC A.10, GLP	Min. 99.0%		Not highly flammable	[redacted] 2007 (A3.10_01); [redacted] 2000 (A3_11-01)
Self-reactive substances and mixtures	UN H.4; non-GLP	99.6%		SADT > 75°C; No exothermic effects	[redacted] 2011 (no BPD-ID)
Pyrophoric liquids	--	--	--	n/a, not a liquid nor gas	--
Pyrophoric solids	Expert Statement, GLP	--	--	No pyrophoric properties	[redacted] 2006 (A3_11-02)

Hazard class / characteristics	Guideline and Method	Purity of the test substance (% (w/w)	Parameter(s)	Results / Waiver	Reference
Self-heating substances and mixtures	Directive 92/69/EEC A.16, GLP	99% 98.7%	--	No self-heating detected up to 400 °C; no spontaneous combustion	[redacted] 2007 (A3.10_01); [redacted] 2000 (A3_11-01)
Substances and mixtures which in contact with water emit flammable gases	Expert Statement, GLP	--	--	No liberation of flammable gases in hazardous amounts upon contact with water.	[redacted] 2006 (A3_11-02)
Oxidising liquids	--	--	--	n/a, not a liquid nor gas	--
Oxidising solids	92/69/EEC A.17, GLP	min. 99.0%		Not oxidising. The tests specified in the CLP Regulation provide additional information on the packing group in case the tested substance turns out to be an oxidising solids. This is not relevant for bronopol as it was classified as "not oxidising solid" based on a negative result in the test according to A.17. Based on the available results for [redacted]/Bronopol ([redacted], 2007, BPD ID A3.10_01 with a burning rate much lower than the reference) it is very likely and thus can be assumed that the results of any tests according to O. 1 and O.3 will also result in a classification as "not oxidising solid". Therefore, the A.17 test is relevant for the CLP classification for bronopol.	[redacted] 2007 (A3.10_01)
Organic peroxides	--	--	--	n/a, not a peroxide	--
Corrosive to metals	--	--	--	n/a, not a liquid	--
Desensitised explosives	--	--	--	n/a, not explosive	--

Hazard class / characteristics	Guideline and Method	Purity of the test substance (% (w/w)	Parameter(s)	Results / Waiver	Reference
Auto-ignition temperature (liquids and gases)	--	--	--	n/a, not a liquid nor gas	--
Relative self-ignition temperature for solids	92/69/EEC A.16, GLP	98.7%	--	No spontaneous combustion	[redacted] 2000 (A3_11-01)
Dust explosion hazard	VDI Guideline 2263, Part 1 DIN EN 14034-1-3:2011 DIN EN ISO 80079-20-2:2016-12	99.6%	--	dust explosion class St 1	[redacted] 2020 (no BPD-ID)

Conclusion:

Bronopol 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 involved in a fire. It is not considered highly flammable, not pyrophoric and does not evolve flammable gases in contact with water, also no self-heating was detected up to 400 °C. Bronopol is not considered to exhibit a danger of explosion in the sense of the directive, but it was found to be detonable and exhibited some thermal explosive properties according to Test Series 1(but is too insensitive for inclusion in Class 1 (explosives) by the United Nations Class 1 acceptance scheme). Bronopol has no oxidizing properties. Crystalline Bronopol 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.

Table 106: Analytical methods for the analysis of the product as such including the active substance, impurities and residues

The biocidal products are identical with the active substance Bronopol. Therefore, the methods submitted for analysis of Bronopol in TGAI are applicable also to Bronopol analysis in biocidal products (see Section A.1.4).

Table 107: Analytical methods for monitoring residues

The methods submitted for analysis of Bronopol residues for the active substance (see Section A.1.4) are applicable to this section

B.2 Efficacy

B.2.1 Efficacy

Table 108: Experimental data on the efficacy of the biocidal product 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
PT2							
Bactericidal, reduction of malodour generating microorganisms	PT 2, chemical toilets	Bronopol	*Escherichia coli* Representative of the enteric bacteria encountered by chemical toilet fluids during use.	Conducted in accordance to adapted ASTM E645-91: Standard Test Method for Efficacy of Microbicides Used in Cooling Systems. The pass criteria for ASTM E645-91 states that an effective level of biocide is that which produces a 99% (2 log) or greater reduction in the test sample counts after the selected exposure time (24 hrs).	A chemical toilet fluid diluted to an in use concentration of 200 ppm Bronopol and a control were inoculated with 0.1 mL of a 24-hour *E. coli* culture to give an approximate inoculum of $1.0x10^8$ cfu/mL. At 0 hrs (control only), 24 hours and 7 days, counts were performed on 1 mL aliquots. Number of replicates: 1. Control sample with sterile distilled water.	Percentage kill was found to be 99.6% after 24 hours and 100% after 7 days. The test established that 200 ppm Bronopol was able to provide protection within a simulated chemical toilet for up to 7 days. The untreated control did not reduce inoculum numbers and failed the test.	B5.10.2_02 Supporting study
Bactericidal, reduction of malodour generating microorganisms	PT 2, chemical toilets	Bronopol	*Escherichia coli* Representative of the enteric bacteria encountered by chemical toilet fluids during use.	A kill test was designed to determine the effect of acid pH on Bronopol activity in an aqueous system over a 24-hour contact time against E. coli.	A [redacted] toilet cleaner containing Bronopol was submitted for testing ([redacted]), and it was determined that the sample did not meet the criteria of 10% cfu/mL reduction after a 24-hour contact time.	After a 24-hour contact period it was determined that pH 2 alone reduced the inocula by $>10^5$ cfu/mL. At pH 7 the aqueous systems containing 400 ppm or more	B5.10.2_03 Supporting study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					A 1000 ppm stock solution of Bronopol was prepared and diluted to give a range of Bonopol concentrations at pH 7 and pH 2. The solutions containing 100, 200, 400, 500 and 1000 ppm Bronopol and a blank control were inoculated with 0.1 mL of $1.0x10^8$ cfu/mL suspension of *E.coli* (24 hour culture). At 0 hrs (control only), 24 hours and 7 days, counts were performed on 1 mL aliquots. Number of replicates: 1 per concentration and pH.	Bronopol reduced the inocula by $>10^5$ cfu/mL after 24 hours. Control at pH 7 shown growth.	
Bactericidal, reduction of malodour generating microorganisms	PT 2, chemical toilets	[redacted] (98% Bronopol)	*Escherichia coli* Representative of the kind of bacteria that may be encountered by a chemical toilet fluid.	An in-house test was conducted to compare the efficacy of Bronopol versus Glutaraldehyde and Formaldehyde.	Deodorant concentrates were formulated to contain 2% non-ionic surfactant, 2% Fragrance, 1.5% buffer solution pH 4.5, 0.25% blue dye and water. Biocides were added at the following concentrations to form the three complete concentrates: 1.3% [redacted] (98% active) 20% Glutaraldehyde (25% active) 81% Formaldehyde (37% active) The three concentrates were then diluted to	Bacterial counts for 200 ppm [redacted] remained at or below 1×10^3 cfu/mL during the test: period. The counts for formaldehyde remained low up to 8 days but rose to 7.8×10^3 cfu/mL by 14 days. 470 ppm glutaraldehyde was unable to retain	B5.10.2_04 Supporting study

<table>
<tr><th>Function</th><th>Field of use envisaged</th><th>Test substance</th><th>Test organism(s)</th><th>Test method</th><th>Test system / concentrations applied / exposure time</th><th>Test results: effects</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>achieve the manufacturer's recommended in toilet biocide concentration ie:
[redacted]: 200 ppm
Glutaraldehyde: 470 ppm
Formaldehyde: 12875 ppm

The use-diluted solutions underwent simulated field testing which included an organic load (tryptic soy broth) and to simulate heavy use an initial challenge of 1 x 10^9 E. coli cfu/mL was added to 3 mL of each use-diluted toilet solution.

Subsequent inoculations of 1 mL of broth containing 1 x 10^5 cfu/mL E. coli were added daily to each toilet solution. The final dilution at the end of the test period of 14 days was 1:4 (one-part diluted toilet solution to four parts bacteria containing waste load).

The test solutions were stored at approximately 100°F (37.8°C) during the test period.

At suitable time intervals during the test period the efficacy of the biocides was determined by carrying out bacterial</td><td>counts below 1 x 10^5 from day 6 onwards.

<table><tr><th rowspan="2">Time</th><th>cfu/mL</th></tr><tr><th>Control</th></tr><tr><td>Day 0</td><td>1.00E+08</td></tr><tr><td>Day 2</td><td>8.80E+08</td></tr><tr><td>Day 6</td><td>4.50E+09</td></tr><tr><td>Day 8</td><td>6.00E+09</td></tr><tr><td>Day 14</td><td>5.00E+09</td></tr></table>
<table><tr><th rowspan="2">Time</th><th>cfu/mL</th></tr><tr><th>[redacted]</th></tr><tr><td>Day 0</td><td>1.00E+08</td></tr><tr><td>Day 2</td><td>9.00E+00</td></tr><tr><td>Day 6</td><td>9.00E+00</td></tr><tr><td>Day 8</td><td>9.00E+01</td></tr><tr><td>Day 14</td><td>1.00E+03</td></tr></table>
<table><tr><th rowspan="2">Time</th><th>cfu/mL</th></tr><tr><th>Glutaraldehyde</th></tr><tr><td>Day 0</td><td>1.00E+08</td></tr><tr><td>Day 2</td><td>9.00E+00</td></tr><tr><td>Day 6</td><td>1.00E+05</td></tr><tr><td>Day 8</td><td>6.70E+07</td></tr><tr><td>Day 14</td><td>3.60E+07</td></tr></table>
<table><tr><th rowspan="2">Time</th><th>cfu/mL</th></tr><tr><th>Formaldehyde</th></tr><tr><td>Day 0</td><td>1.00E+08</td></tr><tr><td>Day 2</td><td>9.00E+00</td></tr><tr><td>Day 6</td><td>9.00E+00</td></tr><tr><td>Day 8</td><td>2.70E+02</td></tr></table></td><td></td></tr>
</table>

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					counts using standard plate count methods.	Day 14 | 7.80E+04	
Bactericidal, reduction of malodour generating microorganisms	PT 2, chemical toilets	[redacted] (10% [redacted]) [redacted] contains a minimum of 95% Bronopol	Enteric bacteria found within raw sewage	Challenge test in accordance with Air Canada Spec Test No. 3125-00002. Counts were made using the APHA Standard Method.	The biocidal product pre-diluted to 0.8 g/L with tap water is called the prime charge. The concentration of Bronopol in the prime charge is 80 ppm. At **time=0** h, it is mixed with fresh raw sewage (FRS: a blend of 1700 mL fresh urine and 200 g of fresh feces mixed and incubated for 24 h at 25°C. This FRS contains ca. 10^9 cfu/mL total bacteria and ca. $2x10^7$ coliform bacteria) in a ratio 1:1.5. The Bronopol concentration in this mixture is **53.3 ppm**. At t=0.5 h, the mixture is split in 2 parts and 1 part is again diluted with FRS to yield a mixture in which the prime charge is diluted 1:2. The Bronopol concentration in this mixture is 40 ppm. At t= 1.0 h, the latter mixture is again split in 2 parts and 1 part is diluted again with FRS to yield a mixture in which the prime charge is diluted 1:5. The Bronopol concentration in	80 ppm Bronopol in the sanitary fluid leads to a strong reduction of bacteria (ca. 4 log reduction in 48 h) and Coliform bacteria (ca. 2 log reduction in 48 h) in the chemical toilet reservoir with respect to a water negative control. Moreover, malodour of the mixture in the reservoir is suppressed to a moderate level, instead of a very strong odour sniffed for the water control after 48 h. The test established that 80 ppm Bronopol (53.3 ppm at time=0) was able to provide protection for up to one hour of simulated intensive use. The total experiment time of 48 h is also representative for many chemical toilet	B5.10.2_05 **KEY STUDY**

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					this mixture is 16 ppm. At t=0 h also a "1:5 water control" is prepared out of 1 part tap water and 4 parts FRS. Each of the 4 mixtures are kept on a shaker at 35°C and after 0, 1, 3, 5, 24 and 48 hours each of these 4 mixtures are sampled and total bacterial count and coliform bacteria are determined. Moreover, for the 1:5 water control and the 1:5 test sample (3x challenged with a final BN concentration of 16 ppm) the odour is evaluated by sniffing. The odour is rated on a scale of 0-4 (0= no odour; 1 = slight odour; 2=moderate odour; 3=strong odour; 4= very strong odour). The water control is rated to 4. Single test. Bacterial numbers were plated out in duplicate.	systems between beginning of use and emptying the system.	
Bactericidal, reduction of malodour generating microorganisms	PT 2, chemical toilets	[redacted]	*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*	Based on DIN EN 1276-2019 Quantitative suspension test (phase 2, step1)	The test solutions containing [redacted] were prepared in hard water containing high loads of protein	Sufficient bactericidal effects (3 log reduction) were observed against all test	B5.10.2_06 Supporting study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			E. coli was chosen as a representative of typical faecal bacteria. *P. aeruginosa* as a representative of the very robust bacterial group of Pseudomonades that can grow in very divers environments due to their flexibility in metabolism. *S. aureus* was tested as a representative for gram-positive bacteria.	Dilution-neutralisation procedure. For odor control, an immediate and complete disinfection isn't necessary, but the biocide must be capable to significantly reduce the number of bacteria that are involved in the formation of malodor. In the present study, it was assumed that a 99.9% reduction (log 3) of bacteria after 24-48 hours in the test suspension is sufficient to control the generation of malodor in chemical toilets.	contamination (dirty conditions) in order to simulate use conditions to a certain level. Test solutions: 0, 2, 12, 25, 50, 100 mg/L [redacted]. The untreated reference sample was based on hard water and protein soiling without [redacted]. Test contact times: 5 min, 1 hour, 24 hours and 48 hours after addition to the contaminated sample. Number of replicates: 2. The 5 min and 1-hour sampling times were chosen to obtain information on short term bactericidal activity. As odor generation is a slow process and chemical toilets are in use for a couple of days usually, 24 hours and 48 hours are the sampling times relevant for odor control.	organisms at 100 mg/l Bronopol after 48 hours. Significant effects against the gram-negative test bacteria *P. aeruginosa* and *E. coli* were already achieved with 50 mg/L Bronopol after contact times of 24 to 48 hours. Requirements of inactivation and validation controls were successfully fulfilled.	
PT11							
Bactericidal, preventing bacterial growth/ removing	PT 11, preservative for liquid-cooling and processing	[redacted] (99% Bronopol)	Mixed aerobic bacterial inoculum containing: *P. aeruginosa*	An in-house test method was used to determine the effectiveness of Bronopol in	The test vehicles chosen were two types of water, sterile water of standard hardness and sterile pond water each dosed	[redacted] dosed at 50 and 100 ppm into pond water and water of standard hardness in	B5.10.2_01 Supporting study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
microbial contaminations in cooling and process waters	systems		*E. aerogenes* *P. fluorescens* *K. aerogenes and* *B. subtilis* Mixed anaerobic bacterial inoculum containing: *Desulphovibrio desulphuricans* subsp. *Desulphuricans NCIB 8307 and Desulphovibrio desulphuricans* subsp. *desulphuricans* NCIB 8301	controlling aerobic and anaerobic bacteria in stored (non potable) water.	separately with [redacted] at levels of 50 and 100 ppm active ingredient (Bronopol) and stored in two different types of container (polypropylene and stainless steel). Aerobic samples were stored in open (uncovered) and closed (covered) conditions at 28±2°C. The open system samples were stored in a temperature controlled incubator, which was lit by 7 x 30 Watt daylight fluorescent tubes to represent natural daylight conditions. Closed system samples were covered and stored in a dark standard laboratory incubator. All anaerobic test systems were stored at 28±2°C in an anaerobic workstation. The samples were dosed with approximately $1.0x10^5$ cfu/mL of the mixed inoculums. The test systems were sampled at 1, 7, 14 and 30 days. After 30 days any systems that showed recovery of 10^3-cfu*mL^{-1} were re-dosed with 10ppm of product and re-sampled	open and closed polypropylene containers will bring about average reductions in cell numbers of >3 logs within 1 to 7-days. This level of cell reduction was maintained or further reduced over the remainder of the test period. [redacted] in pond water at 50 and 100 ppm in closed stainless steel containers initially controlled the microbial challenges although regrowth was evident after 30-days. A subsequent dosing of an additional 10 ppm [redacted] reduced the cell numbers to below 10^3 cfu/mL within 3-days. Although all open stainless steel systems dosed with [redacted] had completely evaporated by the 30-day sampling point, the results	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					according to the test regime. Positive and negative controls were used. The positive control is the water samples unpreserved but innoculated with bacteria and that the negative control the sample water unpreserved and not inoculated. Number of replicates: One per water quality (water of standard hardness and pond water) and storage containment (polypropylene and steel) and storage condition (open storage container and closed storage container).	obtained showed significant reductions of up to >4 log by 14-days. The anaerobic efficacy test results demonstrate that [redacted] dosed at 50 and 100 ppm will bring about a 5 log (>99.99%) reduction in colony forming units per mL within 24 hours which was maintained throughout the test period when compared to the controls. It should be noted that the stainless steel systems had evaporated by the 30-day sampling point, however significant reductions of 5 log (>99.99%) were observed up to 14-days. All results were compared to the controls over the 30-day test period. All positive controls shown growth.	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
						Negative controls did not show any bacterial growth during the 30-days test period.	
Bactericidal, preventing bacterial growth/ removing microbial contaminations in cooling and process waters	PT 11, preservative in air scrubbing / washing units	[redacted] (≥99% Bronopol)	Various bacteria; Organisms not determined	Field Trial Problems were highlighted in a pharmaceutical tablet coating unit. Despite the use of a quaternary ammonium type biocide dosed at 200 ppm twice weekly, high bacterial populations (ranging from 10^4 to 10^6 bacteria per mL) developed rapidly with resulting slimes and foul odours.	In the trial the treatment was changed to [redacted] dosed twice weekly at 40 ppm. Samples of water from the air scrubbing unit were taken at suitable time intervals during the trial and the total viable bacterial count of each sample determined using a pour plate count technique. In total 9 weeks. From on application to the next application: approx. 1 week. No controls and no replicates are reported.	[redacted] controlled bacteria to extremely low levels. Dosing 40 ppm a.s. twice per week gave a 4 to 6 log reduction of bacteria. In many other industrial systems it is not necessary to achieve such low levels and reduced quantities of Bronopol will probably be suitable.	B5.10.2_11 Supporting study
Bactericidal, preventing bacterial growth/ removing microbial contaminations in cooling and process waters	PT 11, preservative in air scrubbing / washing units	[redacted] Bugstick ([redacted] Bugstick) (≥99% Bronopol)	Various bacteria; Organisms not determined	Field Trial; A large hospital was known to have experienced high bacterial counts and considerable fouling in several of its 900 l air washer units.	In an effort to determine the most effective biocide treatment regime, each of four washer units was dosed with a quaternary amine in week one, followed by a mixture of isothiazolinones in week two. A 50 ppm dose of [redacted] was then added, in the form of a solid [redacted] Bugstick	The bacterial counts remained relatively high in weeks 1 and 2 when using quaternary amine or Isothiazolinone as the treatment biocide. In weeks 3 and 4 when the treatment was changed to [redacted] the bacterial	B5.10.2_12 Supporting study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					([redacted] Bugstick), during weeks three and four. Total bacterial counts were determined at suitable time intervals. Controls: No, but effects were compared to treatments with quarternary amine (week 1) and isothiazolinone mixture (week 2).	numbers were significantly reduced. [redacted] controlled bacteria to extremely low levels. Dosing 50 ppm. once per week gave a 2 to 5 log reduction of bacteria.	
Bactericidal, preventing bacterial growth/ removing microbial contaminations in cooling and process waters	PT 11, preservative in cooling systems	[redacted] (20% solution Bronopol)	*Pseudomonas aeruginosa, Legionella pneumophila*	ASTM E645-91 Efficacy of Microbicides used in Cooling Systems. To determine the biocide's efficacy in the presence of an organic soil, the procedure was repeated adding sterile, pulverised yeast cells to the CTW. The pass criterion was a 99% (2 log) reduction in the test sample counts after the selected exposure time.	Sterilised cooling tower water (CTW) was inoculated separately with two test organisms. [redacted] at 0, 25, 50, 100 and 200 ppm (0, 5, 10, 20 and 40 ppm of Bronopol respectively) was added to the CTW. Storage temperature was maintained at water temperature in cooling towers ± 5 ºC: 15 ± 5 ºC for samples without organic soilant. 17 ± 5 ºC for samples with organic soilant. At 24, 48, 72 hours and 7 days after inoculation, the flasks were sampled, plated out into appropriate media and incubated. Number of replicates: 2.	Without organic soilant: [redacted] was very effective against *L. pneumophila*: 25ppm [redacted] (5 ppm Bronopol) had 100% kill at 48 hours). Against *P. aeruginosa:* 200 and 100 ppm [redacted] (40 and 20 ppm Bronopol) had 100% kill at 24 hours up to 14 days. 25 ppm [redacted] (5 ppm Bronopol) had approx. 99.99% kill upto 72 hours then began loosing efficacy at 7 days. At 50 ppm concentration, [redacted] (10 ppm Bronopol) began loosing efficacy at 7 days.	B5.10.2_13 **KEY STUDY** (for *L. pneumophila* only)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					All controls shown growth. The initial counts were higher than 10e5 and control population remained stable during the test period.	With organic soilant: Against *L. pneumophila*, all [redacted] concentrations were highly effective at 24 hours, but only produced 100% kill after 48 hours. No [redacted] concentration tested produced the required 2 log reduction in the numbers of *P. aeruginosa*.	
Bactericidal, preventing bacterial growth/ removing microbial contaminations in cooling and process waters	PT 11, cooling and process waters	[redacted]	Mixed suspension of five bacterial species: Gram-negative: *Pseudomonas aeruginosa, Enterobacter cloacae chloacae, Aeromonas hydrophila* Gram-positive: *Staphylococcus aureus* and *Enterococcus hirae* The tested bacteria are relevant as contaminations of	ASTM 645-18 - Standard Practice for Evaluation of Microbicides Used in Cooling Water Systems. Each test sample was done in triplicate and not in duplicate only. Evaluation was done according to criteria applied in IBRG test methods, *e.g.* PDG 16-007.03	Artificial cooling water composition (in demin. water): $CaCl_2$ (0.861 g/L), Plurafac® LF221 (0.5 g/L) as dispersing agent, Korantin® PP (0.05 g/L) as corrosion inhibitor, yeast extract (5 mg/L), pH adjusted to 8.2. Test concentrations: 0, 0.5, 1, 2, 5, 10, 20 mg/L Test temperature: 30 ± 1 ºC. Exposure time: the efficacy was evaluated by comparing the number of microorganisms recovered from the treated samples 3	Cooling water samples treated with ≥2 mg/L Bronopol showed a clear reduction of the bacterial contamination within the 24 hours testing period, while cooling water samples treated with ≤1 mg/L Bronopol showed an increase of the number of bacteria comparable to the level in the untreated control (bacterial growth increase by a factor of 400 withing 24 hours).	B5.10.2_14 **KEY STUDY**

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
			cooling and process waters.		hours, 5.5 hours and 24 hours after addition to the contaminated artificial cooling water with the number of bacteria in the contaminated untreated control. Number of replicates: 3	After 24 hours, Bronopol at 5 ppm gave a 2 log reduction and Bronopol at 10 and 20 ppm gave almost total kill. Preventive efficacy is demonstrated at the concentration of 2 mg/L Bronopol and curative efficacy could be accepted at 5 mg/L after a contact time of 24 hours. All controls shown growth.	
PT12							
Bactericide, control of bacterial slimes in paper mill systems	PT 12, slimicides in paper mills	Bronopol ([redacted])	*Enterobacter aerogenes* *Pseudomonas aeruginosa*	ASTM E600-91 'Efficacy of Slimicides for the Paper Industries - Bacterial Slime' A 1% pulp slurry containing rosin size was buffered to pH 8 and inoculated with a 10^6 c.f.u./mL mixed inoculum of *Pseudomonas aeruginosa* and *Enterobacter aerogenes*.	The slurry was treated with Bronopol at 10 ppm a.s. The bacterial count was measured initially in controls and at 3, 6 and 24 hours in all the treated samples. 10 ppm Bronopol 24 hours	After 24 hours, Bronopol at 10 ppm a.s. gave a 2 log reduction whereas 50 ppm a.s. gave almost total kill.	B5.10.2_15 Key study

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
Bactericidal, control of bacterial slimes in paper mill systems	PT 12, slimicides in paper mills	Bronopol ([redacted])(minimum 95% Bronopol)	Various bacteria; Organisms not determined	**Laboratory trials:** **1)** Activity in paper process white water.	**1)** Samples of contaminated white water from a paper mill were maintained shaken at 25°C and treated daily with 0, 10, 25 or 50 ppm Bronopol. Each day, prior to biocide addition, samples were taken for bacterial counts and replaced with an equivalent volume of process water containing approximately 10^4 colony forming units (c.f.u.)/mL. Number of replicates is not reported.	**1)** Bronopol [redacted] at 10 ppm a.s. once per day gave a 2 log reduction within 1 day and completely eradicated the bacteria within 3 days, whereas 25 and 50 ppm a.s. completely eradicated the bacteria within 1 2 days. Controls shown growth.	B5.10.2_15 Key study
				2) Activity in thin stock.	**2)** Contaminated thin stock was treated with [redacted] at 0, 10, 15, 20 and 30 ppm. Samples with drawn daily for bacterial counts were replaced equivalent volumes of contaminated water containing approximately 104 c.f.u./mL. Number of replicates is not reported.	**2)** All concentrations gave a 4 log reduction within 1 day. At 10 ppm there was a re-growth and within 4 days it gave a 3 log reduction. [redacted] at 15, 20 and 30 ppm once per day gave a 5 log reduction within 4 days. Controls shown growth.	
				3) Preservation of	**3)** Saples of thick stock	**3)** Microbial counts at the start of the	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				thick stock.	wer shock dose once only with [redacted] and the microbial counts of aerobic bacteria, fungi and sulfate-reducing bacteria (SRB) were recorded. Concentrations tested: 0, 25, 50, 100 and 200 ppm. Number of replicates is not reported.	trial were comparable with the 2-day control counts except for the SRB which were initially ten fold lower. Even the lowest dose of [redacted] gave effective bacterial control up to 2 days, although there was evidence of regrowth at 7 days. All other treatments gave a full 7 days control. On a qualitative level, it was noted that the untreated control rapidly developed a foul, rancid odour, which persisted strongly throughout the trial. None of the treated samples developed any odour during the trial.	
				4) Activity in board process water.	**4)** Samples of contaminated board process water from a mill in Northern England were treated daily with varying levels of [redacted]. Concentrations of 15, 20 and 30ppm daily and	**4)** Treatment levels of 15, 20 and 30ppm daily, and 30ppm and 50ppm on alternate days, were effective as bacteriostats, maintaining the	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
					concentrations of 30ppm and 50ppm on alternate days. Number of replicates is not reported.	aerobic bacterial counts at approximately 10^4 c.f.u./mL over a 4-day period. However, [redacted] at 10ppm was less effective with bacterial counts of 10^5 and 10^7 on days 3 and 4 of the test. In the untreated control, numbers rose to approximately 10^7 c.f.u./mL by day 1 and remained at 10^7 to 10^8 during the test period. All the treatment levels also controlled the growth of yeasts and moulds.	
				5) Activity in an Emulsion Size.	**5)** Samples of a water based emulsion size contaminated with Gram-negative rod shaped bacteria were treated once with [redacted]. Concentrations tested: 0, 100, 150 and 200 ppm. Number of replicates is not reported.	**5)** While bacterial numbers in the untreated size increased, 100ppm [redacted] and higher controlled the contamination and after 4 days no bacteria were detected.	
				6) Activity in pulp slurry according to	**6)** The slurry was treated with [redacted] at 10 and	**6)** According to the ASTM method, an	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				ASTM E600-91: 'Efficacy of Slimicides for the Paper Industries-Bacterial Slime' A 1% pulp slurry containing rosin size was buffered to pH 8 and inoculated with a 10^6 c.f.u./mL mixed inoculum of *Pseudomonas aeruginosa* and *Enterobacter aerogenes*.	50 ppm a.s. The bacterial count was measured initially in controls and at 3, 6 and 24 hours in all the treated samples. Number of replicates: 2.	effective slimicide should give 99% kill (or a 2 log reduction). This was applicable at 24 hours to both 10 ppm (4 log reduction) and 50 ppm (5 log reduction) by 24 hours. Controls shown growth.	
Bactericidal, control of bacterial slimes in paper mill systems	PT 12, slimicide in paper industry	[redacted]	Mixed suspension of five bacterial species: Gram-negative: *Pseudomonas aeruginosa, Enterobacter chloacae, Aeromonas hydrophila* Gram-positive: *Staphylococcus aureus*, *Enterococcus hirae*	ASTM 1839-13 (modified): 1) The number of bacteria used as inoculum was reduced to $5x10^4$ to $5x10^5$ cfu/mL of pulp slurry. 2) [redacted] addition was done 20 hours after inoculation of the pulp slurry and not at the beginning of the test as given in ASTM 1839-13. This allows for the adaption of the bacteria to the paper matrix and mimics a realistic situation in a papermill.	Aqueous cellulose pulp slurries were inoculated with a mixed suspension of the five bacterial species. And allowed to grow for 20 h at 32ºC. [redacted] was added after this initial growth phase. The efficacy was evaluated by comparing the number of microorganisms recovered from the treated samples 3 hours and 24 hours after addition to the contaminated cellulose pulp with the number of bacteria in the untreated control after the same period. Test system: 1% pulp slurries (2/3 pine wood fiber, 1/3 linters)	According to the ASTM method, an effective slimicide should give 99% kill (or a 2 log reduction). Pulp samples treated with 1 mg/kg Bronopol showed a similar bacterial growth characteristic as the control sample (increase by a factor of 15) => 1 mg/kg not sufficiently effective within 24 hours; Pulp samples treated with 10 mg/kg not effective after 3 hrs, but only after 24 hrs (>2 log reduction);	B5.10.2_16 **KEY STUDY**

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				Calculation of bactericidal effects was done comparing bacterial counts of treated and untreated reference samples at the same time respectively. 3) *Aeromonas hydrophila* was introduced as an additional contamination relevant in paper making processes. To cover typical gram-positive contaminations, two gram-positive bacteria were also added to the mixture: *Staphylococcus aureus* and *Enterococcus hirae*. 4) Inoculation was done with a mix of the bacteria. 5) pH adjustment was done by using a pH 5 buffer system and not with sulfuric acid	Number of bacteria in inoculum: $5x10^4$ cfu/mL Test concentrations: 0, 1, 10, 40 mg/kg (ppm) Temperature: 32 ± 2 ºC during the whole test under shaken conditions. Exposure time: 24 hours Number of replicates: 3	For pulp samples treated with 40 mg/kg a slight reduction was already observed after 3 hrs (0.5-1 log reduction) and increased after 24 hrs (>2 log reduction). All controls shown growth.	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test system / concentrations applied / exposure time	Test results: effects	Reference
				to ensure pH stability over the test period. 6) Each test sample was even done in triplicate and not only in duplicate.			

B.2.2 Mode of action

Since the representative biocidal product is identical with the active substance, the information on mode of action is also valid for the product.

B.2.3 Resistance

Since the representative biocidal product is identical with the active substance, the information on resistance is also valid for the product.

B.2.4 Conclusion on efficacy

The evaluation of the summary data provided in support of the efficacy of the representative products, establishes that the products may be expected to be efficacious. Provided data are sufficient at active substance approval stage. However, at product authorisation efficacy studies according to the relevant guidance documents which appropriately simulate the applied in-use situation have to be submitted for organisms representative for the application area.

PT 2:

B5.10.2_02: Supporting study. A diluted chemical toilet fluid was tested for efficacy against *Escherichia coli* according to the ASTM E645-91 adapted method. The chemical toilet fluid containing 200 ppm Bronopol met the pass criteria demonstrating a 99.6% reduction within 24 hours and 100% after 7 days. Nevertheless, the eCA do not consider the results as conclusive since only one replicate has been performed and odor control was not tested.

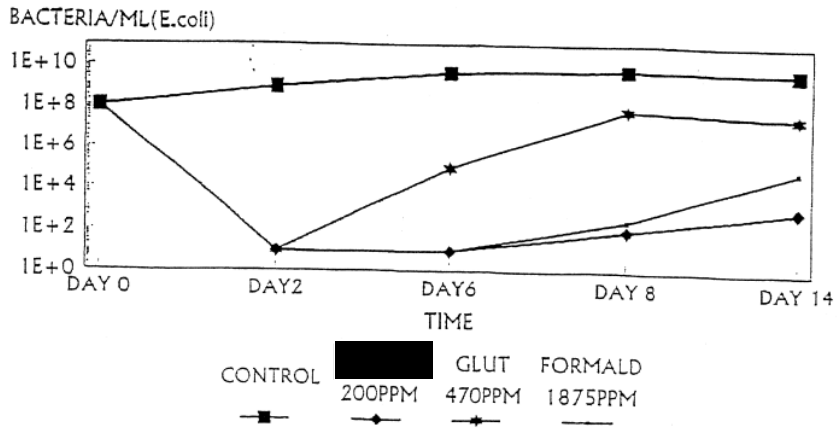
B5.10.2_03: Supporting study. This kill test was designed to determine the effect of acid pH on Bronopol activity in an aqueous system over a 24-hour contact time against *E. coli*. After a 24-h contact period it was determined that pH 2 alone reduced the inocula by $>10^5$ cfu/mL. At pH 7 the aqueous systems containing 400 ppm or more Bronopol reduced the inocula by $>10^5$ cfu/mL after 24 hours.

B5.10.2_04: Supporting study (because odor control was not tested and only a single replicate was performed). Tests were carried out to compare [redacted] (98% Bronopol) against glutaraldehyde and formaldehyde, two most commonly used biocides in chemical toilet deodorants. In use concentrations of the deodorant formulations underwent simulated field testing which included an organic load (tryptic soy broth) and inoculated with *Escherichia coli* to give an initial waste load of $1.0x10^9$ cfu/mL. The test systems were stored at 100 F (37.8 °C) and rechallenged with organic load and bacteria (1 mL of a culture of *E. coli* containing $1x10^5$ cfu/mL) on a daily basis. Bacterial counts were carried out at suitable time intervals to determine the activity of the biocides. [redacted] at an in use concentration of 200 ppm was more effective in controlling bacterial growth than the other two biocides at manufacturers in-use concentrations. It maintained the bacterial count at 1×10^3 cfu/mL or less over the 14-day test period despite daily rechallenge (see tables 109 and figure B5.10.2_04b).

Table 109

Time	cfu/mL			
	Control	Bronopol 200 ppm	Glutaraldehyde 470 ppm	Formaldehyde 1875 ppm
Day 0	1.00E+08	1.00E+08	1.00E+08	1.00E+08
Day 2	8.80E+08	9.00E+00	9.00E+00	9.00E+00
Day 6	4.50E+09	9.00E+00	1.00E+05	9.00E+00
Day 8	6.00E+09	9.00E+01	6.70E+07	2.70E+02
Day 14	5.00E+09	1.00E+03	3.60E+07	7.80E+04

Figure B5.10.2_04b:



B5.10.2_05: KEY STUDY. [redacted] (10% W/W [redacted]. [redacted] contains a minimum of 95% Bronopol) was tested for suitability as an antimicrobial aircraft sanitary fluid using the Air Canada Specification Test, number 3125-00-002 (see attached appendix in Doc IIIB):

At time zero, 200 milliliters of "fresh raw sewage is added to 400 millilitres of sanitary fluid (prime charge: 80 ppm Bronopol) to make a prime charge dilution ratio of 1:15. A tap water control shall also be prepared with 4 parts fresh raw sewage to 1 part tap water to obtain 1:5 dilution.

NOTE: The "fresh raw sewage" samples shall be made up of 1700 milliliters of fresh urine and 200 grams of fresh faeces. The resultant mixture shall then be uniformly blended following by incubation for 26 hours at 25°C. After incubation and before additions are made to the sanitary fluid prime charge, the mixture shall be filtered through a pledget of glass wool to remove detritus.

At 30 minutes, the 1:1.5 dilution sample is divided into two equal portions (300 mL each) and 100

milliliters of raw sewage is added to one of these portions to obtain a 1:2 dilution.

At 60 minutes, the 1:2 dilution sample is again divided into two equal portions (200 mL each) and 300 milliliters of raw sewage is added to one of these portions to obtain a 1:5 dilution.

Following the addition of raw sewage as in the above method at zero, 30 and 60 minutes the concentration of Bronopol at each dilution point was:

0 hours (1:1.5 dilution): 53.3 ppm Bronopol.

30 mins (1:2 dilution): 40.0 ppm Bronopol.

60 mins (1:5 dilution): 16.0 ppm Bronopol.

Conclusion: with a "prime charge" concentration of 0.8 g/l (80 ppm Bronopol), and with repeated loading of organic waste up to a dilution of 1:5 with raw sewage the sanitary fluid [redacted] passed the test. 80 ppm Bronopol in the sanitary fluid (equivalent to 53.3 ppm active substance after the first addition of sewage) leads to a strong reduction of bacteria (ca. 4 log reduction in 48 h) and Coliform bacteria (ca. 2 log reduction in 48 h) in the chemical toilet reservoir with respect to a water negative control. This equates to the control of sewage related bacterial growth with 53.3, 40.0 and 16.0 ppm active substance. Moreover, malodour of the mixture in the reservoir is suppressed to a moderate level, instead of a very strong odour sniffed for the water control after 48 h (see table 110). Since the aim of the use of Bronopol in the case of chemical toilets is not the curative effect, but the control of malodour producing bacteria for odor reduction, we consider that this field study fairly accurately reflects the conditions of actual use and odour reduction is demonstrated.

Table 110:

DILUTION RATIO		TOTAL MICROBIAL & COLIFORM COUNT TEST TIME (HRS)					
		0	1	3	5	24	48
1 : 1 . 5	TBC	3.6 X 10^4	5.2 X 10^4	1.48 X 10^4	1.08 X 10^4	3.04 X 10^4	5.76 X 10^4
	Coliforms	14.0 X 10^4	28.0 X 10^4	1.0 X 10^4	9.2 X 10^3	1.12 X 10^4	1.44 X 10^4
1 : 2	TBC	14.8 X 10^4	5.2 X 10^4	1.2 X 10^4	1.32 X 10^4	6.08 X 10^4	7.5 X 10^4
	Coliform	10.4 X 10^4	12.8 X 10^4	1.92 X 10^4	3.2 X 10^3	1.84 X 10^4	6.6 X 10^4
1 : 5	TBC	12.8 X 10^4	4.0 X 10^4	5.6 X 10^4	1.68 X 10^4	4.24 X 10^4	7.1 X 10^4
	Coliform	12.8 X 10^4	14.4 X 10^4	2.72 X 10^4	8.0 X 10^4	3.36X 10^4	3.8 X 10^4
Control	TBC	2.11 X 10^8	3.5 X 10^8	2.9 X 10^8	3.7 X 10^8	3.1 X 10^8	4.4 X 10^8
1 : 5	Coliform	5.0 X 10^6	5.9 X 10^6	5.1 X 10^6	6.9 X 10^6	5.7 X 10^6	7.0 X 10^6

TBC = Total Bacterial Count

ODOUR EVALUATION: 2-4

B5.10.2_06: The present study, based on DIN EN 1276, was performed to demonstrate the efficacy of [redacted] (Bronopol) as an appropriate biocide for odor control in chemical toilets (PT 2). The test solutions containing [redacted] were prepared in hard water containing high loads of protein contamination (dirty conditions) in order to simulate use conditions to a certain level.

For odor control, an immediate and complete disinfection isn't necessary, but the biocide must be capable to significantly reduce the number of bacteria that are involved in the formation of malodour. As odor generation is a slow process and chemical toilets are in use for a couple of days usually, 24 hours and 48 hours are the sampling times relevant for odor control. Bactericidal effects (3 log reduction) were observed against the gram-negative and the gram-positive test bacteria at 100 mg/L [redacted] after 48 hours. Significant effects against the gram-negative test bacteria *P. aeruginosa* and *E. coli* were achieved already with 50 mg/L [redacted] after contact times of 24 to 48 h.

CONCLUSION PT2:

The use of chemical toilets is usually associated with accumulation of microorganism and odor issues caused by microbial activities. Therefore, Bronopol is intended to be used in chemical toilets to reduce the number of microorganisms responsible for malodor generation.

There are currently no standard tests available in the Guidance for this use. Among the studies presented, the eCA considers that the selected key study B5.10.2_05 is that best reflects the actual conditions of the chemical toilets. This test shows that Bronopol produces sufficient bacterial reduction at concentrations of 16 to 80 ppm with single application in chemical toilets. Based on the data submitted, the product is expected to be efficacious, but more consistent in use tests must be submitted at product authorization stage.

PT 11:

B5.10.2_01: Supporting study. Efficacy data has been generated to demonstrate the effectiveness of Bronopol at controlling the growth of aerobic and anaerobic water borne bacteria in stored (non potable) water. The Bronopol product tested was [redacted] (nominally 99% Bronopol). The test vehicles chosen were two types of water, sterile water of standard hardness and sterile pond water each dosed separately with [redacted] at levels of 50 and 100 ppm active ingredient (Bronopol) and stored in two different types of container (polypropylene and stainless steel). Throughout the 30-day test period, [redacted] at levels of between 50–100 ppm product with subsequent additional dosing of 10 ppm when required, demonstrated effective microbial control against the aerobic and anaerobic test organisms.

B5.10.2_11: Supporting study (no controls and no replicates are reported). In this field test Bronopol was dosed twice weekly over a period of 9 weeks at 40 ppm in water of a wet scrubbing unit. The results shown that Bronopol controlled bacteria to extremely low levels. Dosing 40 ppm a.s. twice per week gave a 4 to 6 log reduction of bacteria.

B5.10.2_12: Supporting study. Field test for determination of the efficacy of a Bronopol treatment at 50 ppm against bacteria colonisation in air scrubbing units. The bacterial counts remained relatively high in weeks 1 and 2 when using quaternary amine or Isothiazolinone as the treatment biocide. In weeks 3 and 4 when the treatment was changed to [redacted] the bacterial numbers were significantly reduced. [redacted] controlled bacteria to extremely low levels. Dosing 50 ppm. once per week gave a 2 to 5 log reduction of bacteria.

B5.10.2_13: KEY STUDY (for *L. pneumophila* only). Test performed according ASTM E645-91 to determine the biocide's efficacy in the presence and in the absence of an organic soil. Sterilised cooling tower water (CTW) was inoculated separately with two test organism (*L. pneumophila* and *P. aeruginosa*). [redacted] (20% Bronopol) at 0, 25, 50, 100 and 200 ppm was added to the CTW. Under clean conditions, [redacted] in a concentration of 25 ppm (5 ppm Bronopol) is deemed to be effective in reducing bacteria colonisation in cooling tower water. With the presence of organic soilant efficacy is limited to *L. pneumophila* where all tested concentrations reduced the number of viable bacteria by >99% after 48 hours.

Although only 2 replicates have been performed, we consider it acceptable within the framework of the active substance approval. However, more efficacy test in real conditions should be submitted at product authorization stage.

B5.10.2_14: KEY STUDY. Test performed according to ASTM 645-18 to demonstrate the efficacy of [redacted] as a preservative for liquid cooling and processing waters. The bacteria chosen are relevant as contaminations of cooling and process waters. It was shown that the untreated cooling water (untreated control) is susceptible to bacterial growth. Bacterial counts increased from 10^5 cfu/mL to 10^7 cfu/mL within 5.5 hours of incubation period and further on to $5x10^7$ cfu/mL within 24 hours (see table 111). In contrast, cooling water samples treated with 1 mg/l [redacted] or below showed an increase of the number of bacteria during the 24 hours testing period. Cooling water samples treated with 2 mg/L [redacted] showed a slight reduction of the bacterial contamination within the 24 hours testing period. After 24 hours, Bronopol at 5 ppm gave a 2 log reduction of bacteria and 10 and 20 ppm gave almost total kill (see table 112). The study shows a clear dose response of bacterial reduction in the artificial cooling water. Preventive efficacy is demonstrated at

the concentration of 2 mg/L Bronopol and curative efficacy could be accepted at 5 mg/L after a contact time of 24 h.

Table 111: colony forming units (cfu/mL) of bacterial mix

[redacted]		Cfu/ml			
	Replicate	0h	3h	5.5h	24h
0 mg/l untreated control	1	1.0E+05	6.3E+05	1.4E+07	3.5E+07
	2	1.3E+05	7.9E+05	1.5E+07	4.4E+07
	3	1.3E+05	8.5E+05	1.5E+07	6.7E+07
0.5 mg/l	1	1.2E+05	2.4E+05	6.8E+05	5.4E+07
	2	1.2E+05	2.7E+05	8.9E+05	5.1E+07
	3	1.0E+05	2.3E+05	1.1E+06	4.4E+07
1 mg/l	1	1.1E+05	1.6E+05	2.1E+05	2.9E+07
	2	1.0E+05	1.3E+05	2.3E+05	2.6E+07
	3	1.3E+05	1.4E+05	2.4E+05	3.7E+07
2 mg/l	1	1.3E+05	8.6E+05	9.8E+04	4.6E+04
	2	1.4E+05	1.2E+05	1.1E+05	5.3E+04
	3	1.3E+05	1.1E+05	9.6E+04	3.8E+04
5 mg/l	1	1.3E+05	5.9E+04	3.5E+04	1.5E+03
	2	1.4E+05	5.7E+04	5.2E+04	1.2E+03
	3	1.3E+05	7.3E+04	4.6E+04	1.2E+03
10 mg/l	1	1.2E+05	3.0E+04	2.1E+04	5.0E+01
	2	1.4E+0	5.0E+04	2.5E+04	1.2E+02
	3	9.3E+04	3.5E+04	2.3E+04	1.0E+01
20 mg/l	1	1.4E+05	3.3E+04	2.3E+04	1.0E+01
	2	1.4E+05	2.8E+04	1.9E+04	<1.0E+01
	3C	9.8E+04	2.8E+04	2.0E+04	2.0E+01

Table 112: Bactericidal activity against bacterial mix (mean values, three replicates).

	Cfu/ml			
	0h	3h	5.5h	24h
0mg/l untreated control	1.2E+05	7.6E+05	1.5E+07	4.9E+07
0.5 mg/l	1.2E+05	2.5E+05	8.8E+05	5.0E+07
1.0 mg/l	1.1E+05	1.4E+05	2.2E+05	3.1E+07
2.0 mg/l	1.3E+05	1.0E+05	1.0E+05	4.6E+04
5.0 mg/l	1.3E+05	6.3E+04	4.4E+04	1.3E+03
10.0 mg/l	1.2E+05	3.8E+04	2.3E+04	6.0E+01
20.0 mg/l	1.3E+05	3.0E+04	2.0E+04	1.5E+01

CONCLUSION PT11:

The key in-use test B5.10.2_14 with cooling water formulation showed that water samples treated with 2 mg/l [redacted] or more showed a significant reduction of the bacterial contamination within the 24 hours testing period. After 24 hours, Bronopol at 5 ppm gave a 2 log reduction of bacteria and 10 and 20 ppm gave almost total kill.

Test B5.10.2_13 determines the efficacy of [redacted] (20% Bronopol) in the presence and in the absence of an organic soil against *Legionella.* At 25 ppm (5 ppm Bronopol) and higher produced 100% kill after 48 hours. Howerver, as it was performed only whith 2 replicates, at product authorisation stage studies according to the relevant guidance documents which appropriately simulate the applied in-use situation have to be submitted.

Consequently for the purpose of the efficacy assessment of the Bronopol as PT11, the application rate of 2 ppm of the representative product and one application every 24 hours is validated for preventive use against bacteria and curative efficacy is demonstrated at 5 mg/L after a contact time of 24 hours.

PT 12:

B5.10.2_15: Key study. Conditions for microbial growth are likely to vary considerably in each paper mill and for these reason, it is difficult to prescribe the most effective treatment regime for a mill before trials have been carried out. Nevertheless, these simulated use laboratory tests demonstrate the efficacy of [redacted] (minimum 95% Bronopol) under typical treatment conditios. The results of the tests indicate that the biocidal product shows efficacy from 10 ppm onwards. According ASTM E600-91, 10 ppm applied in the pulp sample demontrates a curative efficacy against bacteria after a contact time of 24 hours.

B5.10.2_16: KEY STUDY. It was shown that the untreated cellulose pulp substrate (untreated control) is susceptible for bacterial growth. Bacterial counts increased from 10^5 cfu/mL to 10^6 cfu/mL within 20 hours of incubation period (see table 113), prior to the addition of [redacted]. A slight additional growth was observed over the following 24 hours. The results with the cellulose pulp show that [redacted] is effective as a slimicide at concentrations of 10mg/kg (w/w) and higher (see table 114). The study shows a clear concentration dependent bacterial reduction in the paper pulp (see table 115).

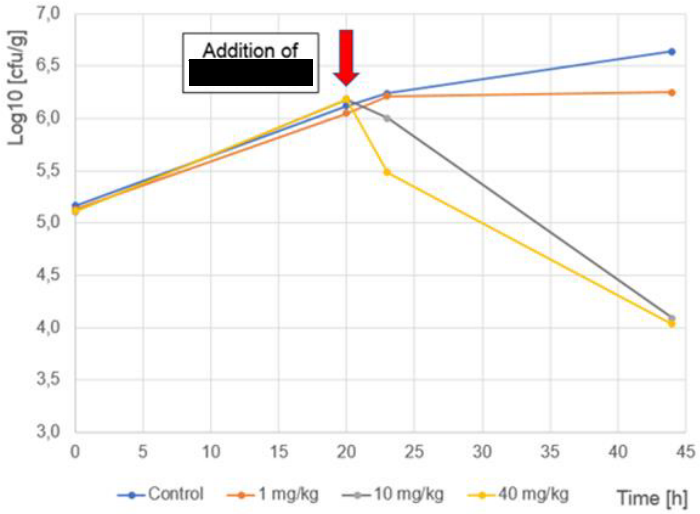
Table 113: colony forming units (cfu/mL) of bacterial mix in the pulp samples.

		Cfu/ml before [redacted] addition		Cfu/ml after [redacted] addition	
[redacted]	Replicate	0h	20h	3h	24h
0mg/kg untreated control	A	1.5E+05	1.4E+06	1.2E+06	1.6E+06
	B	1.5E+05	1.8E+06	2.0E+06	3.4E+06
	C	1.4E+05	9.0E+05	2.2E+06	1.5E+06
1mg/kg	A	1.1E+05	1.2E+06	1.0E+06	1.3E+06
	B	1.3E+05	1.3E+06	1.9E+06	3.1E+06
	C	1.7E+05	9.0E+05	2.3E+o6	1.4E+06
10mg/kg	A	1.5E+05	1.3E+06	1.1E+06	1.8E+04
	B	1.0E+05	1.4E+06	9.8E+05	1.9E+04
	C	1.4E+05	1.9E+06	9.3E+05	5.5E+03
40mg/kg	A	1.5E+05	2.7E+06	5.4E+05	9.6E+03
	B	1.2E+05	1.1E+06	1.5E+05	1.1E+04
	C	1.2E+05	1.2E+06	3.5E+05	1.2E+04

Table 114: Bactericidal activity against bacterial mix (mean values, three replicates).

	Growth before addition of [redacted]		Growth after addition of [redacted]	
[redacted]	0h	20h	3h	24h
0mg/kg untreated control	1.5E+05	1.4E+06	1.8E+06	2.2E+06
			% reduction relative to untreated control	
1mg/kg	1.4E+05	1.1E+06	1.9%	10.8%
10mg/kg	1.3E+05	1.5E+06	43.4%	99.3%
40mg/kg	1.3E+05	1.7E+06	80.7%	99.5%

Table 115: log cfu/mL (mean values) *vs*. time



CONCLUSION PT12:

The key in-use test with aqueous cellulose pulp slurries showed that the product [redacted] provided bacterial control (>2 log reduction) in use dilutions containing 10 and 40 ppm Bronopol.

For the purpose of the efficacy assessment of the Bronopol as PT12, the application rate of 10 ppm and higher of the representative product and one application every 24 hours is validated for preventive and curative use against bacteria.

B.3 Human exposure assessment

General Remarks

The assessment of occupational exposure towards Bronopol as disinfectant, preservative, slimicides and algaecide not intended for direct application to humans or animals is based on information provided by the Participants. In the absence of human exposure data, the exposure estimation to Bronopol is based on the selected models and default values from the Biocides Human Health Exposure Methodology (BHHEM 2015) along with HEEG recommendations and the Guidance on the Biocidal Products Regulation Volume III Human Health - Assessment & Evaluation (Parts B+C) Version 4.0 December 2017.

If no appropriate models are available in the BHHEM, surrogate models are chosen and a justification is provided.

The proposed tiered approach for human exposure assessment is applied as follows. In several cases it is considered not to be appropriate to calculate a "reasonable worst case" exposure (Tier 1) according to the guidelines. The dermal absorption of Bronopol in humans is well established as outlined below. Assuming no protection by the human skin (as proposed for Tier 1 estimates) is considered not to be reasonable. For all of the following calculations the established dermal absorption figure for humans is applied. Despite the fact that protective measures could be supposed to be carefully observed in a professional environment, a Tier 1 is proposed as a worst case. Then, personal protective equipment will be assumed to be worn as second scenario (Tier 2).

Unless otherwise specified, a default penetration value of 10% for gloves and clothing was assumed, which is in accordance with HEEG Opinion on "Default protection factors for protective clothing and gloves" (when potential hand exposure data are available, a factor of 10 -90 % reduction of exposure by gloves manufactured from appropriate material- can be used as a reasonable and conservative default value to convert the potential to actual hand exposure when using appropriate gloves: MOTA v6, 4.2.9.9 HEEG Opinion 9). On the other hand, if data on exposure inside protective gloves is available, these will be used for exposure assessment (MOTA v6, 4.2.9.2 HEEG Opinion 2).

Where exposure is calculated based on empirical data (database models provided in the Biocides Human Health Exposure Methodology (BHHEM 2015) along with HEEG recommendations), these data are applied in agreement with the recommendations given by the guidelines as follows: In case of continuous (chronic) exposure scenarios the typical exposure is calculated based on the 75%-ile of the data. The 95%-ile is considered to represent the typical case when recommended by applicable guidelines. Where 95%-iles are not given, the maximum values are used instead.

Local dermal risk assessment

According to the criteria of the Regulation 1272/2008, Bronopol is proposed to be classified as a severe eye irritant category 1 (H318), an irritant to skin category 2 (H315) and an irritant to respiratory tract category 3 (H335). As AECs can be set, quantitative and qualitative assessment of local effects will be performed in Section B.

B.3.1 PT02

MG/PT	Field of use envisaged	Likely concentration at which a.s. will be used
Major fields of use		
Used for disinfection of chemical toilets.	Disinfectants to control potentially pathogenic microorganisms and odour generating microorganisms in chemical toilets	0.0016 – 0.008 % w/w Bronopol 16 – 80 ppm

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.

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.

B.3.1.1. Identification of main paths of human exposure towards active substance from its use in biocidal product

Three groups of humans may be potentially exposed to the representative product:

- Professional users that apply and dispose of products containing the representative product.
- Non-professional users handling products formulated from the representative product such as concentrates for portable toilets, caravans and vehicles.
- The general public that may be incidentally exposed after the product has been used.

Handling and application of the representative product as well as end use products containing the biocidal product in a professional or amateur environment can result in direct exposure via skin contact or via inhalation. Contamination by ingestion should not occur under usual working practices as long as a minimum of hygiene standards are observed. The oral route is therefore not included as a potential direct route for exposure during the use of products which contain biocidal product. Exposure via the environment is another potential route, which is however rather an indirect than a direct one. It is considered to be of no relevance for the working environment and therefore not addressed in this section.

In the following, the procedures relevant for the handling and application of the representative product as well as the products containing Bronopol are described and potential sources of direct exposure identified. Indirect exposure scenarios are described in the corresponding section.

Summary table: relevant paths of human exposure							
Exposure path	**Primary (direct) exposure**			**Secondary (indirect) exposure**			
		Professional use	**Non-professional use**		**Professional use**	**General public**	**Via food**
Inhalation		Yes	Yes		Yes	Yes	No
Dermal		Yes	Yes		Yes	Yes	No
Oral		No	No		No	No	No

Bronopol, the active substance (a.s.) of the representative product, is manufactured outside the EU, however, the manufacturing plant is ISO compliant and Government Approved. The Rapporteur assumes that the production/formulation is performed in conformity with national and European occupational safety and health regulations

The representative product is identical to the active substance Bronopol, 2-Bromo-2-nitropropane-1, 3-diol. In the exposure assessment presented below, the following stages have been considered.

PRIMARY EXPOSURE

- Professional use of formulations of end use products containing Bronopol: chemical toilets.

Professional users will be exposed to lower amounts of biocidal product in formulated products.

- Non-professional: Using sanitary products for application into chemical toilets

SECONDARY EXPOSURE

- Indirect exposure. The general public may be exposed incidentally and indirectly to use of products containing bronopol by the following pathways, for example: dermal contact with treated surfaces or inhalation exposures to use of formulated products containing bronopol.

B.3.1.2. List of scenarios

Summary table: scenarios			
Scenario number	**Scenario (*e.g.* mixing/ loading)**	**Primary or secondary exposure Description of scenario**	**Exposed group (*e.g.* professionals, non-professionals, bystanders)**
2	Application	Primary exposure - Application of final end-use product to the waste holding tank and emptying of collecting tanks after usage	Professionals
3	Application	Primary exposure - Emptying of collecting tanks after usage	Professionals
4	Application	Primary exposure - Application of the final end-use product to chemical toilet. Manual application	Non-professionals
5	Application	Primary exposure - Emptying of the chemical toilet waste tank	Non-professionals
6	Post-application	Secondary exposure - General public in contact to spillage during the use of chemical toilets	General public
7	Post-application	Secondary exposure - Inhalation of volatilized residues	General pubic
7a	Post-application	Secondary exposure - Exposure to vapour to formaldehyde	General pubic

B.3.1.4. Professional exposure

Chemical toilet products for adding to the flushing fluid or waste water tanks and may be used for the maintenance of portable chemical toilets or chemical toilets in caravans/campers. Active substance concentration is up to 10% in liquid chemical toilet products and up to 30% in products sold in water-soluble bags/sachets.

Exposure of professionals is expected during the application of the chemical toilet product. Activities also include the replacement of empty disinfectant containers with full ones and connecting them to the flushing tank of the chemical toilet. Further contact with the active substance may be possible while emptying the waste container of chemical toilets.

Scenario 2- Semi-automated application into chemical toilets (Addition of biocidal product concentrate to chemical toilet)

Description of Scenario 2
This scenario covers the semi-automated application of sanitary products into chemical toilet by professional workers. This task involves replacing chemical toilet product containers and collecting tanks, removing and capping of empty container and opening and connecting fresh container with feeding line. Professionals may use liquid products containing up to 10% active substance, whereas solid products contained in water soluble bags containing up to 30% a.s. Water soluble bags act as a barrier based on common understanding. As the bag remains intact until the bag/sanitary product is given into the sewer tank, the substance Bronopol cannot cross this bag. Water soluble bags are usually made from water soluble polymers having the appearance of a common plastic film. Therefore, it was concluded that exposure during the manual application is considered negligible. For this scenario the recommendation for Professional users exposed to during the mixing and loading operations during manual or automated addition for liquids is applied in line with HEAdhoc

recommendation no. 6. Though it is recommended to use this model with care, Mixing and loading model 7 is considered the most appropriate for repeated loading and/or semi-automated transfer/loading of biocidal products as Mixing and loading model 5 is rather considered for simple loading (*e.g.* 1 bag per day) and other models do not provide relevant/reliable indicative inhalation and/or dermal exposure values.

Therefore, and in line with the suggestions from HEAdhoc recommendation no. 6, Mixing and loading model 7 (TNsG part 2 p.142 (corrected)) was taken into account. The indicative values from this model are considered to be a worst case, considering that this task is mainly a "closed" mixing and loading, as replacing of containers does not involve any removing of the active substance from the containers, but only closing/opening containers as well as connecting them to the feeding line.

In the absence of any specific information about the length of time required to apply the biocidal product it is assumed as a default that the operator takes 10 minutes per day to apply the biocidal product. This is based on default information from the TNsG excel database on human exposure.

In Tier 2 PPE are considered.

	Parameters	Value / Units	Justification / Source
Tier 1	Weight fraction of active substance (Bronopol)	10%	Applicant's data
	Weight fraction of active substance (Formaldehyde)	0.004%	Worst case
	Body weight	60 kg	Recommendation no. 14, 2017
	Task Duration Bronopol	10 min/day	TNsG
	Task Duration Formaldehyde	192 min/day	TNsG
	Indicative dermal exposure without gloves (Bronopol)	101 mg/min	Recommendation no. 6, 2020
	Indicative dermal exposure without gloves (Formaldehyde)	138 mg/min	Recommendation no. 6, 2020
	Indicative inhalation exposure (Bronopol)	0.94 mg/m3	Recommendation no. 6, 2020
	Indicative inhalation exposure (Formaldehyde)	22 mg/m^3	Recommendation no. 6, 2020
	Inhalation rate	1.25 m3/h	Recommendation no. 14, 2017
	Dermal Absorption (Bronopol)	10%	Section B5.2, Section A.3.1.1.2.
	Dermal Absorption (Formaldehyde)	50%	Section B5.2, Section A.3.1.1.2.
Tier 2	Indicative dermal exposure under gloves (Bronopol)	1.01 mg/min	Recommendation no. 6, 2020
	Indicative dermal exposure under gloves (Formaldehyde**)**	1.38 mg/min	Recommendation no. 6, 2020

Calculations for Scenario 2

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 2 (Bronopol)	1 / none	3.26E-04	1.68E-01	1.69E-01
Scenario 2 (Formaldehyde)	1 / none	5.87E-05	8.83E-03	**8.83E-03**
Scenario 2 (Bronopol)	2 / PPE	3.26E-04	1.68E-03	2.01E-03
Scenario 2 (Formaldehyde)	**2 / PPE**	**5.87E-05**	8.**83E-05**	1.47E-04

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m³)**	**Estimated dermal concentration (ppm)**
Scenario 2 (Bronopol)	1 / none	1.96E-03	100000 ppm
Scenario 2 (Formaldehyde)	1 / none	3.52E-04	40 ppm
Scenario 2 (Bronopol)	2 / PPE	1.96E-03	100000 ppm
Scenario 2 (Formaldehyde)	2 / PPE	**3.52E-04**	40 ppm

Scenario 3 – Exposure during the emptying of chemical toilet waste tank.

Description of Scenario 3
Though in the professional setting the mixing and loading task is considered to be of (semi-) automated nature, for this scenario, the manual addition of the sanitary product into the sewage tank of the chemical toilet using liquid products containing up to 10% active substance is calculated as a worst case. Solid products contained in water soluble bags containing up to 30% a.s. are not considered for this scenario, as no exposure is expected during the application of these products. The emptying of the waste tank of the chemical toilet is considered to be covered by this scenario. As the concentration of the active substance is significantly lower after dilution in the chemical toilet (to a maximum of 100 ppm) simple pouring of the waste task is expected to result in comparable or lower dermal exposure and negligible inhalation exposure due to the low vapour pressure of Bronopol. In line with ECHA's Biocides Human Health Exposure Methodology Guidance (October 2015), the ConsExpo Factsheet for Disinfectants to potential exposure scenarios/pattern of use for disinfectants for chemical toilets was used as this might be applicable also for professional users. Default data for mixing and loading of Disinfectant for chemical toilets from the Disinfectant Products Fact Sheet is applied taking into account a higher frequency for professionals. It is considered that a professional processes 4-6 toilets per hour over a working shift of 8 hours, hence 48 toilets per day as a worst case assumption.

	Parameters	**Value / Units**	**Justification / Source**
Tier 1	Weight fraction of active substance (Bronopol)	0.01%	Worst-case product
	Body weight	60 kg	Recommendation 14, 2017
	Frequency	48/ day	Applicant´s data
	Respiration volume	1.25 m^3 air/hour	Recommendation 14, 2017
	Exposure duration	1.33 min	Disinfectant products fact sheet (ConsExpo)
	Product amount	500 g	Disinfectant products fact sheet (ConsExpo)
	Room volume	1 m^3	Disinfectant products fact sheet (ConsExpo)
	Ventilation rate	0.6 /h	Disinfectant products fact sheet (ConsExpo)
	Mass transfer coefficient	10 m/h	Disinfectant products fact sheet (ConsExpo)
	Release area	0.002 m^2	Disinfectant products fact sheet (ConsExpo)
	Exposed area	215 cm^2	Disinfectant products fact sheet (ConsExpo)
	Dermal Absorption	50 %	Guidance on Dermal Absorption (EFSA, 2017)
Tier 2	PPE (protection gloves)	10%	HEEG opinion 9.

Calculations for Scenario 3

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 3	1 / none	2.27E-10	4.00E-04	4.00E-04
Scenario 3	2 / PPE	2.27E-10	4.00E-05	4.00E-05

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: Local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m³)**	**Estimated total dermal concentration (ppm)**
Scenario 3	1 / none	1.02E-08	100 ppm

Combined scenarios

Summary table: combined systemic exposure from professional uses				
Scenarios combined	**Tier**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenarios [2, 3]	Tier 1	3.26E-04	1.69E-01	1.69E-01
Scenarios [2, 3]	Tier 2 / PPE	3.26E-04	1.72E-03	2.05E-03

B.3.1.5. Non-Professional exposure

Also, non-professionals use chemical toilet disinfectant up to 30% Bronopol. Consumer exposure is due to pouring liquid disinfectant as it is assumed that during use of a chemical toilet the private user is not exposed to the disinfectant.

Exposure is divided in two different tasks:

- Application of liquid concentrate.
- Emptying of chemical toilet waste tank.

Scenario 4 - Manual application into chemical toilets

Description of Scenario 4
Scenario for non-professional application of liquid concentration into chemical toilets: For the estimation non-professionals applying sanitary products into chemical toilets, similar assumptions were made as for professionals during the application. Non-professionals might the manual pour of the sanitary products into the sewage tank of the chemical toilet (as so-called pre-charge) using liquid products containing up to 10% active substance or solid products contained in water soluble bags containing up to 30% a.s. Water soluble bags act as a barrier based on common understanding. As the bag remains intact until the bag/sanitary product is given into the sewer tank, the substance Bronopol cannot cross this bag. Water soluble bags are usually made from water soluble polymers having the appearance of a common plastic film. Therefore, it was concluded that exposure during the manual application is considered negligible. In line with ECHA's Biocides Human Health Exposure Methodology Guidance (October 2015), the ConsExpo Factsheet for Disinfectants to potential exposure scenarios/pattern of use for disinfectants for chemical toilets was used. Default data for mixing and loading of Disinfectant for chemical toilets from the Disinfectant Products Fact Sheet is applied taking into account a higher frequency of 7 times per year. This is based on the assumption that the chemical toilet is used for a period of 4 weeks a year in accordance with the ConsExpo Fact Sheet. Every 4 days after emptying the waste tank, the user adds the stated amount of disinfectant to the content of the waste tank.

	Parameters	Value / Units	Justification / Source
Tier 1	Weight fraction of active substance (Bronopol)	10%	Applicant's data.
	Body weight	60 kg	Recommendation 14, 2017
	Frequency	7/year	Disinfectant products fact sheet (ConsExpo)
	Respiration volume	1.25 m^3 air/hour	Recommendation 14, 2017
	Exposure duration	1.33 min	Disinfectant products fact sheet (ConsExpo)
	Product amount	500 g	Disinfectant products fact sheet (ConsExpo)
	Room volume	1 m^3	Disinfectant products fact sheet (ConsExpo)
	Ventilation rate	0.6 /h	Disinfectant products fact sheet (ConsExpo)
	Mass transfer coefficient	10 m/h	Disinfectant products fact sheet (ConsExpo)
	Release area	0.002 m^2	Disinfectant products fact sheet (ConsExpo)
	Exposed area	215 cm^2	Disinfectant products fact sheet (ConsExpo)
	Dermal Absorption	10 %	Section B5.2, Section A.3.1.1.2.

Calculations for Scenario 4

Systemic effects

Summary table: systemic exposure from non - professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 4	**1 / none**	4.22E-10	1.**67E-02**	1.67E-02

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: Local exposure from non- professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m^3)**	**Estimated total dermal concentration (ppm)**
Scenario 4	1 / none	9.14E-07	100000 ppm

Scenario 5 - Emptying of the chemical toilet waste tank

Description of Scenario 5
The emptying of the waste tank of the chemical toilet is considered to be covered by the above scenario 3 (professional). As the concentration of the active substance is significantly lower after dilution in the chemical toilet (to a maximum of 100 ppm) simple pouring of the waste task is expected to result in comparable or lower dermal exposure and negligible inhalation exposure due to the low vapour pressure of Bronopol.

B.3.1.6. Secondary exposure of the general public excluding dietary exposure

In the following, those secondary exposure scenarios are assessed as proposed by the Guidelines on Human Exposure to Biocidal Products. Among these, there are scenarios which may be considered to represent worst cases for all of the relevant exposure routes. Based on the use pattern of the biocidal product for the different uses envisaged by the Applicant indirect inhalation exposure to Bronopol could be expected.

The secondary exposure assessed in the following refers to the inhalation exposure of adults to chemical toilets disinfectant during the time spent in atmospheres or housings containing these systems. There might be many more conceivable secondary exposure scenarios which however are assumed to represent lower exposure risks compared to the described worst case scenarios and are therefore not additionally assessed.

Scenario 6 – Use of chemical toilets

Description of Scenario 6			
Secondary exposure of the general public could be expected during the use of the chemical toilet after application of chemical toilet products. In this scenario, consumers may be exposed to the maximum amount of Bronopol in waste water which is 100 ppm (0.01%). Based on the ConsExpo Disinfectant Products Fact Sheet, it is assumed that during use of a chemical toilet (*e.g.* in bus airplanes or caravans…) the private user is not exposed to the disinfectant. Also, the ECHA's Biocides Human Health Exposure Methodology Guidance (October 2015) provides information that toilets are designed to minimise biocide aerosol generation or splashing during use. Moreover, due to the low vapour pressure of Bronopol, no relevant inhalation exposure is expected for people using toilets. As a worst case, exposure to Bronopol for users of mobile toilets is estimated using ConsExpo, considering the event of spillage during flushing the toilet (5 times per day).			
	Parameters	**Value / Units**	**Justification / Source**
Tier 1	Weight fraction of active substance (Bronopol)	0.01%	Applicant's data.
	Body weight	23.9 kg	Recommendation 14, 2017
	Frequency	5/day	Applicant´s data
	Respiration volume	1.25 m^3 air/hour	Recommendation 14, 2017
	Exposure duration	1.33 min	Disinfectant products fact sheet (ConsExpo)
	Product amount	500 g	Disinfectant products fact sheet (ConsExpo)
	Room volume	1 m^3	Disinfectant products fact sheet (ConsExpo)
	Ventilation rate	0.6 /h	Disinfectant products fact sheet (ConsExpo)
	Mass transfer coefficient	10 m/h	Disinfectant products fact sheet (ConsExpo)
	Release area	0.002 m^2	Disinfectant products fact sheet (ConsExpo)
	Exposed area	215 cm^2	Disinfectant products fact sheet (ConsExpo)
	Dermal Absorption	50 %	Guidance on Dermal Absorption (EFSA, 2017)

Calculations for Scenario 6

Summary table: systemic exposure from non - professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 6	**Tier 1 / none**	4.82E-12	1.05E-04	1.05E-04

See calculation spreadsheets available in Appendix II.

Scenario 7 - Inhalation of volatilized residues of Bronopol

Description of Scenario 7

Professional and general public may be exposed to volatilised residues from treated chemical toilets. However, based on the document, HEEG opinion 13 on Assessment of Inhalation Exposure of volatilised biocide active substance, it might not be necessary to calculate the exposure to volatilised residues:

- For bronopol :

  $0.328*(Mw*Vp)/AEL_{long\text{-}term} = (0.328*200*0.0051)/0.07 = 4.8 > 1$

  Remark: the mw (molecular weight), vp (vapour pressure) and $AEL_{long\text{-}term}$ come from the Section A.1.3.

The result of this equation is upper than 1 for Bronopol. The exposure to volatilised residues indoor cannot be considered negligible for workers and general public for the active substance.

Exposure is assessed with a dilution of product at 0. 01% (100 ppm).

Inhalation of volatilised residues is assessed with RIVM ConsExpo Web, version 1.1.0 "Exposure to vapour" considering the exposure to vapor during 8 h as it is a professional exposure (worst case), a dilution at 0.01% of bronopol applied in a room of 1 m^3 with a ventilation rate of 0.6/h.

The day of treatment, the professional will not stay in the room for 8 hours. However, he could enter into the room for control task. Anyway, inhalation exposure has been estimated for 8 hours as worst case.

To our case:

- The product largely consists of a single component (water) and therefore the mol weight matrix should be roughly equal to 18 g/mol for water.
- In a worst case basis, the solution (in use concentration) required to treat a room of 1 m^3 with 500 grams for a liquid with a density of 1 g/mL.

This scenario of control task after treatment will be combined with the exposure during application and exposure during touching a treated surface.

	Parameters 1	**Value / Units**	**Justification / Source**
Tier 1	Weight fraction of active substance	0.01%	Applicant's data.
	Body weight: Adult Child Toddler Infant	 60 kg 23.9 kg 10 kg 8 kg	Recommendation 14, 2017
	Inhalation rate: Adult Child Toddler Infant	 16 m^3/24h 12 m^3/24h 8 m^3/24h 5.4 m^3/24h	Recommendation 14, 2017
	Vapour pressure (Vp):	0.0051 Pa	Section A.1.3
	Molecular weight (Mw):	200 g/mol	Section A.1.3
	Gas constant (R)	8.31451 $J.mol^{-1}.K^{-1}$	HEEG opinion no. 13
	Temperature (T)	293 K	HEEG opinion no. 13
	Parameters 2	**Value / Units**	**Justification / Source**
Tier 1	Weight fraction of active substance	0.01%	Applicant's data.
	Frequency	7/year	Disinfectant products fact sheet (ConsExpo)
	Respiration volume	1.25 m^3 air/hour	Recommendation 14, 2017
	Exposure duration	8 h	Assumption (worst case)
	Product amount	500 g	Disinfectant products fact sheet (ConsExpo)
	Room volume	1 m^3	Disinfectant products fact sheet (ConsExpo)

	Ventilation rate	0.6 /h	Disinfectant products fact sheet (ConsExpo)
	Mass transfer coefficient	10 m/h	Disinfectant products fact sheet (ConsExpo)
	Release area	0.002 m^2	Disinfectant products fact sheet (ConsExpo)
	Emission duration	8 h	Assumption (worst case)
	Inhalation Absorption	100 %	Default value
	Molecular weight matrix	18 g/mol	Disinfectant products fact sheet (ConsExpo)
	Application temperature	20 ºC	Disinfectant products fact sheet (ConsExpo)
	Vapour pressure	5.10E-03 Pa	Section A.1.3
	Molecular weight (Mw):	200 g/mol	Section A.1.3

Calculations for Scenario 7

Summary table: systemic exposure from non - professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 7	**Tier 1 / none**	5.59E-11	--	5.59E-11

See calculation spreadsheets available in Appendix II.

Combined scenarios

Summary table: combined systemic exposure from non-professional uses				
Scenarios combined	**Tier**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenarios [4, 5, 6, 7]	Tier 1	4.83E-10	1.72E-02	1.72E-02

Scenario 7a – Exposure to vapour to formaldehyde

Description of Scenario 7a			
Professional and general public may be exposed to vapours from treated chemical toilets. The inhaled dose of formaldehyde is calculated using the inhalation exposure to vapour model implemented in ConsExpo. Parameters were applied to the model as follows.			
Tier 1	**Parameters**	**Value / Units**	**Justification / Source**
	Vapour pressure (Vp):	0.0215 Pa	Section A.1.3
	Molecular weight (Mw):	30 g/mol	Section A.1.3
	Weight fraction of active substance	0.004%	Applicant's data.
	Frequency	240/year	Disinfectant products fact sheet (ConsExpo)
	Respiration volume	1.25 m^3 air/hour	Recommendation 14, 2017
	Exposure duration	10 min	Assumption (worst case)
	Product amount	500 g	Disinfectant products fact sheet (ConsExpo)
	Room volume	1 m^3	Disinfectant products fact sheet (ConsExpo)
	Ventilation rate	0.6 /h	Disinfectant products fact sheet (ConsExpo)
	Mass transfer coefficient	6820 m/min	Disinfectant products fact sheet (ConsExpo)
	Release area	0.002 m^2	Disinfectant products fact sheet (ConsExpo)
	Inhalation Absorption	100 %	Default value

	Molecular weight matrix	218 g/mol	Disinfectant products fact sheet (ConsExpo)

Calculations for Scenario 7a

Summary table: systemic exposure from non - professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 7a	**Tier 1 / none**	2.53E-07	--	2.53E-07

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: Local exposure from non- professional uses			
Exposure scenario	**Tier/PPE**	**Mean event concentration (mg a.s./m³)**	**Estimated total dermal concentration (ppm)**
Scenario 7a	**1 / none**	7.30E-05	40 ppm

Summary table: Local exposure from non- professional uses			
Exposure scenario	**Tier/PPE**	**Mean concentration on day of exposure (mg a.s./m³)**	**Estimated total dermal concentration (ppm)**
Scenario 7a	**1 / none**	5.07E-07	40 ppm

B.3.1.7. Dietary exposure

No dietary exposure is anticipated.

Information of non-biocidal use of the active substance

Summary table of other (non-biocidal) uses			
	Sector of use	**Intended use**	**Reference value(s)**
1.	Veterinary use	- Control fungal infections in fin fish	No MRL established[1].
2.	Human use	- Excipient	No threshold established[2].
3.	Plant protection products	- Fungicide - Bactericide	Not Approval as active substance in accordance with Council Directive 91/414/EEC[3]. Default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg 396 / 2005.
4.	Cosmetic products	- Preservative	Maximum concentration of 0.1 % of BNP to avoid formation of nitrosamines[4].

[1] EMEA/MRL/ 791/01-FINAL June 2001 COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS – BRONOPOL. COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010R0037-20230323).

[2] 22 November 2019 EMA/CHMP/302620/2017 Rev. 2* (*Rev. 2 includes an update of boric acid) - Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668) [https://www.ema.europa.eu/en/annex-european-commission-guideline-excipients-labelling-package-leaflet-medicinal-products-human].

[3] Commission Regulation (EC) No 2076/2002 of 20 November 2002 extending the time period referred to in Article 8(2) of Council Directive 91/414/EEC and concerning the non-inclusion of certain active substances in Annex I to that Directive and the withdrawal of authorisations for plant protection products containing these substances [https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32002R2076].

[4] REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (recast) [https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1223]

Estimating Livestock Exposure to Active Substances used in Biocidal Products

No livestock exposure is anticipated.

Estimating transfer of biocidal active substances into foods as a result of professional and/or industrial application(s)

No further transfer of the biocidal active substance into foods is anticipated.

Estimating transfer of biocidal active substances into foods as a result of non-professional use

No further transfer of the biocidal active substance into foods is anticipated.

B.3.1.8. Exposure associated with production, formulation and disposal of the biocidal product

Production and formulation are addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

B.3.1.9. Combined residential scenarios

None anticipated.

B.3.2. PT11

MG/PT	Field of use envisaged	Likely concentration at which a.s. will be used
Major fields of use		
Cooling water preservative, to control microbial induced damage to equipment /pipe work and the proliferation of potentially pathogenic microorganisms during cooling processes	Preservatives for liquid-cooling and processing systems	10% to 30% % w/w Bronopol (100000 – 300000 ppm) Direct application :100%

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.

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.

B.3.2.1. Identification of main paths of human exposure towards active substance from its use in biocidal product

Different groups of humans may be potentially exposed to the representative product:

- Professional users that apply and dispose of products containing the representative product.
- Non-professional exposure is not envisaged.
- The general public that may be incidentally exposed after the product has been used.

Handling and application of the representative product as well as end use products containing the biocidal product in a professional or amateur environment can result in direct exposure via skin contact or via inhalation. Contamination by ingestion should not occur under usual working practices as long as a minimum of hygiene standards are observed. The oral route is therefore not included

as a potential direct route for exposure during the use of products which contain biocidal product. Exposure via the environment is another potential route, which is however rather an indirect than a direct one. It is considered to be of no relevance for the working environment and therefore not addressed in this section.

In the following, the procedures relevant for the handling and application of the representative product as well as the products containing Bronopol are described and potential sources of direct exposure identified. Indirect exposure scenarios are described in the corresponding section.

Summary table: relevant paths of human exposure							
Exposure path	**Primary (direct) exposure**			**Secondary (indirect) exposure**			
		Professional use	**Non-professional use**		**Professional use**	**General public**	**Via food**
Inhalation		Yes	No		Yes	Yes	No
Dermal		Yes	No		Yes	Yes	No
Oral		No	No		No	No	No

Bronopol, the active substance (a.s.) of the representative product, is manufactured outside the EU, however, the manufacturing plant is ISO compliant and Government Approved. The Rapporteur assumes that the production/formulation is performed in conformity with national and European occupational safety and health regulations.

The representative product is identical to the active substance Bronopol, 2-Bromo-2-nitropropane-1, 3-diol. In the exposure assessment presented below, the following stages have been considered.

PRIMARY EXPOSURE

- Professional use of biocidal product as preservative in liquid cooling. Professional users will be exposed to lower amounts of biocidal product in formulated products.
- Non-professional use of biocidal products is not envisaged.

SECONDARY EXPOSURE

- Indirect exposure of professionals that are intentionally exposed to residual the representative product from contact with process water or through cleaning out or inspecting systems and machinery.
- Secondary incidental exposures to diluted the representative product may occur from windage of water droplets from the cooling tower site. The main route of exposure is via inhalation for this scenario.

B.3.2.2. List of scenarios

Summary table: scenarios			
Scenario number	**Scenario (*e.g.* mixing/ loading)**	**Primary or secondary exposure Description of scenario**	**Exposed group (*e.g.* industrials, professionals, non-professionals, bystanders)**
2.	Application	**Primary exposure.** Biocidal product dilution transferred to the sump.	Professionals
3.	Post-application exposure	**Primary exposure.** Exposure towards residues during cleaning of the dispensing pumps.	Professionals
4.	Post-application exposure	**Primary exposure.** Exposure towards residues during system inspection and monitoring	Professionals
5.	Post-application exposure	**Primary exposure.** Exposure towards residues during cleaning of the fouled systems	Professionals
6.	Post-application exposure	**Primary exposure.** Exposure during disposal of waste	Professionals
7.	Post-application exposure	**Secondary exposure.** Secondary exposure -.Exposure to aerosols	Bystanders

B.3.2.4. Professional exposure

Primary exposure to the biocidal product can be expected during the application as cooling water preservative, relevant for professionals. The biocidal product may be applied directly or, alternatively, as pre-mix into the water matrix to be preserved. The activities include the replacement of empty Bronopol containers with full ones, maintenance work and monitoring tasks, *i.e.* taking water samples for the analysis of microbial contamination.

Scenario 2 – Application.

Description of Scenario 2
After the representative producthas been transferred to the sump, primary user exposure is unlikely to occur, as it is a closed system where worker access is limited. The recirculation systems are highly automated and mechanized. Therefore, the potential for occupational exposure during this phase is considered negligible and will not be assessed. There may be potential exposure to the diluted fluid in use during maintenance and cleaning activities (systems are drained and cleaned annually). In addition, there may be potential exposure during routine inspection (servicing and treatment).

Scenario 3 - Cleaning dispensing pumps

Description of Scenario 3
Maintenance of dosing pumps is conducted by water treatment service personnel and requires cleaning of these items before dismantling. Dispensing pumps are cleaned by service company workers during dosing system drum change outs (professional judgment). For the exposure assessment, it is considered that pump cleaning is the worst-case scenario compared to other post-application activities such as periodic sampling and cleaning a fouled

system. During the cleaning of dispensing pumps, water treatment service professionals may be exposed to the biocidal product. The pumps should be rinsed before cleaning, generally by pumping a sufficient volume of water.

Exposure potential is predominantly to the hands and body, resulting from accidental touching of contaminated surfaces.

Inhalation exposure is considered negligible compared to the dermal exposure considering the volatility of the substance, the short duration of the exposure, the small quantity of product handled and the low potential for aerosol formation. .

No relevant model was found in TNsG (2002) to estimate the exposure rate associated with this scenario.

After the representative product has been transferred to the sump primary exposure to users is unlikely to occur. This is due to the representative product being present in a closed system where worker access is limited. There may be potential exposure to diluted in-use fluid during maintenance and cleaning activities (the systems are drained and cleaned out every year). In addition there may be potential exposure during routine inspection (checking and treatment). Workers can be exposed to diluted biocidal products, *i.e.* up to 5 ppm a.s. at maximum

It is assumed that the dispensing pumps are cleaned by water treatment service professionals during routine dosing system drum change maximum exposure duration 20 min per day.

In line with HEAdhoc recommendation no. 4, no inhalation exposure is assumed during this scenario.

	Parameters	Value / Units	Justification / Source
Tier 1	Weight fraction of active substance (Bronopol)	0.0005%	Applicant's data.
	Body weight	60 kg	Recommendation 14, 2017
	Dermal absorption	50%	Guidance on Dermal Absorption (EFSA, 2017)
	Exposure frequency	20 min/day	Recommendation 4, 2014
	Potential body exposure	19.28 µL/min	Recommendation 4, 2014
	Potential hand exposure	35.87 µL/min	Recommendation 4, 2014
Tier 2	Gloves penetration factor	10%	HEEG Opinion 9

Calculations for Scenario 3

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 3	1 / none	--	4.60E-05	4.60E-05
Scenario 3	2/ PPE	--	1.91E-05	1.91E-05

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m^3)**	**Estimated dermal concentration (ppm)**
Scenario 3	1 / none	--	5 ppm
Scenario 3	2 / PPE	--	5 ppm

Scenario 4 - System inspection and monitoring

Description of Scenario 4
System inspection and consistent monitoring are essential for efficient operation of cooling towers. Routine testing of the cooling water is conducted via a dip slide to monitor for microbial contamination. This scenario is covered in detail in the cleaning dispensing pumps scenario. The exposure potential when handling dip slides to monitor for microbial contamination is anticipated to be far less than those for the cleaning dispensing pumps.

Scenario 5 - Cleaning fouled systems

Description of Scenario 5
Open and closed recirculating cooling water systems are cleaned either once or twice a year by water treatment service professionals. The process involves draining the water from the system, physically or chemically cleaning the system and shock dosing the system with a higher concentration of the biocidal product. During the cleaning of fouled systems, water treatment service professionals may be exposed to the process water containing up to 5 ppm of BNP. "

	Parameters	Value / Units	Justification / Source
Tier 1	Weight fraction of active substance (Bronopol)	0.0005%	Applicant's data.
	Body weight	60 kg	Recommendation 14, 2017
	Dermal absorption	50%	Guidance on Dermal Absorption (EFSA, 2017)
	Exposure frequency	360 min	Recommendation 4, 2014
	Potential body exposure	19.28 µL/min	Recommendation 4, 2014
	Potential hand exposure	35.87 µL/min	Recommendation 4, 2014
Tier 2	Gloves penetration factor	10%	HEEG Opinion 9

Calculations for Scenario 5

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 5	1 / none	--	8.27E-04	8.27E-04
Scenario 5	2/ PPE	--	3.43E-04	3.43E-04

See calculation spreadsheets available in Section D.

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m^3)**	**Estimated dermal concentration (ppm)**
Scenario 5	1 / none	--	5 ppm
Scenario 5	2 / PPE	--	5 ppm

Scenario 6 - disposal of waste

Description of Scenario 6
Bleed-off water from cooling towers and drain-down (blow-down) waste are discharged to the main drainage. Due to the closed nature of these drainage systems, human exposure to bleed-off and/or blow-down waste water is not anticipated.

Scheduled maintenance is conducted on open and closed recirculating cooling systems once or twice a year by specialized companies. These water treatment companies handle the discharged water to prevent its emission into the environment (RIVM, 2003). Potential worker exposure while draining recirculating cooling systems was addressed in detail in the post application scenario for cleaning fouled systems.

Drums containing biocidal products are typically recycled and returned to the supplier for re-use (TNsG, 2002).This scenario is covered in detail in the mixing/loading scenario. The exposure potential when handling empty concentrate drums is anticipated to be far less than those for the sump loading phase.

B.3.2.5. Non-Professional exposure

Application of the biocidal product is assessed for professional exposure only

B.3.2.6. Secondary exposure of the general public excluding dietary exposure

- Indirect exposure of professionals that are intentionally exposed to residual the representative product from contact with process water or through cleaning out or inspecting systems and machinery.
- Secondary incidental exposures to diluted the representative product may occur from windage of water droplets from the cooling tower site. The main route of exposure is via inhalation for this scenario.

There might be many more conceivable secondary exposure scenarios which however are assumed to represent lower exposure risks compared to the described worst case scenarios and are therefore not additionally assessed.

Scenario 7 - Exposure to aerosols

Description of Scenario 7

Professional and general public may be exposed to the drift in the proximity of the cooling towers. There is no model in TNsG for assessing exposure to the aerosol part of the cooling tower system. The ESD on PT 11 gives the following parameters for different type of cooling towers

	Cooling towers			Unit
	Large	Medium	Small	
Recirculating flow rate	9000	100	10	m3/h
Loss by evaporation or drift with drift eliminators	0.01%	0.01%	0.01%	
Water drift flux	0.9	0.01	0.001	m3/h
	0.25	2.78E-03	2.78E-04	L/sec
	250	2.77777778	0.27777778	g/sec
	250000	2777.77778	277.777778	mg/sec
Active ingredient concentration in water (%)	0.00050%			
Active ingredient flux (Fa.i) (mg/sec)	1.25	0.0138889	0.00138889	mg/sec

*According to the ESD, the loss by drift and evaporation (1%) can be reduced by a 100 factor using drift eliminators. So only 0.01% of the recirculating flow is lost.

Considering the exit surface of the tower and the exit velocity of the drift, the dilution volume of the drift can be calculated at the exit of the system.

In combination with the rejected quantity of active substance, a concentration of the substance in the air, just at the exit of the installation, can be assessed with the following equation :

Cair (mg/m3) = Fa.i. (mg/s) / [Sexit (m²) * Vdrift (m/s)]

Where:
Cair (mg/m3): active ingredient concentration at the exit of the tower
Fa.i. (mg/s): active ingredient drift flux
Sexit (m²): exit surface of the tower
Vdrift (m/s): drift velocity at the exit of the tower

Considering that the C_{air} is equal to the long term AEC of 3.6 mg a.i./m^3 (maximum air concentration) and a constant exit velocity of 1 m/s in the lower band of exit velocities of drift from cooling towers (worst-case approach), the minimum surface area of the exit of the cooling tower to reach the dilution necessary to have a C_{air} equal or below to the AEC (reverse scenario) can be derived :

S_{exit} (m²) = $F_{a.i.}$ (mg/s) / [C_{air} (mg/m^3) * V_{drift} (m/s)]

Applying above parameters, S_{exit} can be calculated for the 3 different types of cooling towers:

	Large cooling towers	Medium cooling towers	Small cooling towers
S exit (m2)	0.347	0.00386	0.00039

To verify if S_{exit} of "real" cooling tower are greater than those calculated above, a comparison was made for small, medium and large models with cooling towers from the catalogue of one of the main fabricant of cooling tower[14,15] :

Cooling tower model	Corresponding type of cooling tower	Exit surface (m²)	Maximum calculated Exit surface (m²)
FXVD closed cooling tower	Small/medium	13.69 to 18.49	0.00386
FXV-E closed cooling tower	Small/medium	6.76 to 12.96	0.00386
HFL closed cooling tower	Small/medium	3.87 to 13.32	0.00386
HXI closed cooling tower	Small/medium	2.88 to 6.48	0.00386
PFE/PTE opened/closed cooling tower	Small/medium	4.15 to 10.75	0.00386
S3000-D opened cooling tower*	Small/medium	5.27 to 14.32	0.00386
Lens Noroxo Plant cooling tower **	Large	120	0.347

*normal water flow rate: 108-1008 m^3/h
** normal water flow rate : 3000 m^3/h

The small/medium cooling towers proposed by the applicant have exit surfaces (2.88 to 18.49 m^2) greater than the minimum calculated surface (0.00386 m^2) required to have a C_{air} equal or below to the AEC based on the characteristics of the ESD. Therefore, no unacceptable risk is expected for small/medium cooling tower.

[14] Baltimore Air Coil: http://www.baltimoreaircoil.eu/products
[15] INERIS report February 2004 « Evaluation de la dispersion atmosphérique d'aérosols potentiellement contaminés dans la région de Lens »

The large cooling towers proposed by the applicant have exit surfaces of 120 m^2. This value is lower than the minimum calculated exit surface (0.347 m^2) to have a C_{air} equal or below to the AEC based on the characteristics of the ESD.

For the Lens Noroxo Plant cooling tower (large cooling tower), normal water flow rates was available (3000 m^3/h) and the following drift a.i. concentration was calculated (considering 0.0005% of a.i) as such:

C_{air} (mg/m^3) = $F_{a.i.}$ (mg/s) / [S_{exit} (m^2) * V_{drift} (m/s)]

Where:
$F_{a.i.}$ = 0.416 mg/s
S_{exit} = 120 m^2
V_{drift} = 1 m/s

For more details on the calculations, see Excel data sheet.

	Drift a.i; concentration	Unit
Lens Noroxo Plant cooling tower (Based on normal water flow rate)	0.00347	mg a.i./m^3

The calculated drift a.i. concentration is below to the long term AEC value of 3.6 mg a.i./m^3. The risk is thus considered acceptable for secondary exposure to the drift of large cooling towers.

Overall, the risk is considered acceptable for secondary exposure to the drift of all kind of cooling towers (small/medium/large).

Calculations for Scenario 7

Summary table: systemic exposure from non - professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 7	**Tier 1 / none**	3.47E-3	--	3.47E-3

See calculation spreadsheets available in Appendix II.

B.3.2.7. Dietary exposure

No dietary exposure is anticipated.

Estimating Livestock Exposure to Active Substances used in Biocidal Products

No livestock exposure is anticipated.

Estimating transfer of biocidal active substances into foods as a result of professional and/or industrial application(s)

No further transfer of the biocidal active substance into foods is anticipated.

Estimating transfer of biocidal active substances into foods as a result of non-professional use

No further transfer of the biocidal active substance into foods is anticipated.

B.3.2.8. Exposure associated with production, formulation and disposal of the biocidal product

Production and formulation are addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

B.3.2.9. Combined residential scenarios

None anticipated

B.3.3. PT12

MG/PT	Field of use envisaged	Likely concentration at which a.s. will be used
Major fields of use		
Slimicide, to control microbially induced damage to plant / equipment, pipework during industrial and cooling processes	Slimicides	10% to 30% % w/w Bronopol (100000 – 300000 ppm) Direct application :100%

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.

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.

B.3.3.1. Identification of main paths of human exposure towards active substance from its use in biocidal product

Two group of humans may be potentially exposed tothe representative product:

- Professional users that apply and dispose of products containing The representative product
- Non-professional exposure is not envisaged,

Handling and application of the representative product as well as end use products containing the biocidal product in a professional or amateur environment can result in direct exposure via skin contact or via inhalation. Contamination by ingestion should not occur under usual working practices as long as a minimum of hygiene standards are observed. The oral route is therefore not included as a potential direct route for exposure during the use of products which contain biocidal product. Exposure via the environment is another potential route, which is however rather an indirect than a direct one. It is considered to be of no relevance for the working environment and therefore not addressed in this section.

In the following, the procedures relevant for the handling and application of the representative product as well as the products containing Bronopol are described and potential sources of direct exposure identified. Indirect exposure scenarios are described in the corresponding section.

Summary table: relevant paths of human exposure					
Exposure path	**Primary (direct) exposure**		**Secondary (indirect) exposure**		
	Professional use	**Non-professional use**	**Professional use**	**General public**	**Via food**
Inhalation	Yes	No	Yes	No	No
Dermal	Yes	No	Yes	No	No
Oral	No	No	No	No	Yes

The representative product is identical to the active substance Bronopol, 2-Bromo-2-nitropropane-1, 3-diol. In the exposure assessment presented below, the following stages have been considered.

PRIMARY EXPOSURE

- Professional use of biocidal product as slimicide used in the paper industry.
- Non-professional use of biocidal products is not envisaged.

SECONDARY EXPOSURE

- Indirect exposure. Professionals may be exposed incidentally and indirectly to use of products containing the representative product by the following pathways, for example: dermal and hand-to-mouth oral contact with treated surfaces or inhalation exposures to the representative product following use of formulated products.
- Secondary incidental exposure scenario in PT 12 uses is identified for consumers as a consequence of Bronopol migration from treated paper to food (wrapped in paper treated with Bronopol). The main route of exposure is via ingestion for this scenario.

B.3.3.2. List of scenarios

Summary table: scenarios			
Scenario number	**Scenario**	**Primary or secondary exposure Description of scenario**	**Exposed group**
2.	Post-application: cleaning	**Primary exposure:** Post-application - cleaning of the dispensing pumps	Professionals
3.	Post-application: water sampling	**Primary exposure:** Post application - process water sampling	Professionals
4.	Post-application: maintenance	**Primary exposure:** Post application - process equipment maintenance and RO systems	Professionals
5.	Post-application: disposal of waste	**Primary exposure:** Post-application - disposal of waste	Professionals
6.	Post-application from papermills	**Secondary exposure** from papermills: vapour phase [6a], aerosol phase [6b and 6c]	Professionals
7.	Post-application from papermills	**Secondary exposure** from papermills: contact with paper	Professionals

B.3.3.4. Professional exposure

Exposure occurs during the application of the biocidal product – thereby, the biocidal product may be applied directly or, alternatively, as pre-mix into the water circuit to be preserved. The activities include the replacement of empty Bronopol containers with full ones and taking water samples for the analysis of microbial contamination.

Scenario 2 – Primary exposure - Post-application: cleaning dispensing pumps

Description of Scenario 2
During the cleaning of dispensing pumps, professionals may be exposed to the diluted biocidal product (up to 30%). During the cleaning of dispensing pumps, water treatment service professionals may be exposed, as a very worst-case, to the biocidal product containing up to 30% of Bronopol. More realistically, the pumps should be rinsed before cleaning, generally by pumping a sufficient volume of water. If correctly done, it can be considered that the rinsing leads to a dilution of the product by a factor of 100 at least. After rising, the a.s. concentration will be 0.03%. According to the CAR, the closest model found in the BEAT database (2008) is 'Cleaning of spray equipment', which includes rinsing and rubbing (with paper, rag or brush) tasks, which are similar in cleaning dispensing pumps or fouled system. The indicative exposure values for dermal exposure are as follows: - 35.8 mg/min for hands; - 19.2 mg/min for body. Inhalation is considered negligible because of the low volatility of the substance and the short duration of the exposure. Gloves and coated coverall are taken into consideration in Tier 2. A total exposure duration of 20 minutes/day was taken into account (4 x 5 min/day) following TNsG 2002 (TP.02) [recommendation no.4].

	Parameters	Value / Units	Justification / Source
Tier 1	Weight fraction of active substance (Bronopol)	30%	Worst-case product Applicant's data.
	Body weight	60 kg	Recommendation 14. 2017
	Dermal absorption	50%	Guidance on Dermal Absorption (EFSA. 2017)
	Exposure frequency	20 min/day	Recommendation 4. 2014
	Potential body exposure	19.28 µL/min	Recommendation 4. 2014
	Potential hand exposure	35.87 µL/min	Recommendation 4. 2014
Tier 2	Gloves penetration factor	10%	HEEG Opinion 9
	Concentration of active ingredient after rinsing	0.3%	Dilution factor of 100
	Coated coverall penetration factor	20%	HEEG opinion 9

Calculations for Scenario 2

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 2	1 / none	--	5.52E-01	5.52E-01
Scenario 2	2 / PPE	--	3.72E-03	3.72E-03

See calculation spreadsheets available in Appendix II

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m³)**	**Estimated dermal concentration (ppm)**
Scenario 2	1 / none	--	300000 ppm
Scenario 2	2 / PPE	--	3000 ppm

Scenario 3 – Post application: process water sampling

Description of Scenario 3
Routine testing of the process water is conducted to monitor for microbial contamination. The TNsG (2002) for PT 12.01 (Slimicides for paper pulp) suggests that sampling for microbial counting and examination involve transient hand contact with process water; however. no guidance is provided for the duration and/or frequency of this task. For a similar task involving plant workers inspecting and testing diluted in-use fluid. as described in the TNsG (2002) for PT 11.02 (Preservatives used in recirculating cooling systems). a frequency of once per week and a duration of 2 minutes/sample is suggested. Workers can be exposed to diluted biocidal products. *i.e.* up to 10 ppm a.s. at maximum. The duration for sampling process water of 10 minutes has been chosen (1 exposure event). The indicative exposure values for dermal exposure are as follows: - 35.8 mg/min for hands; - 19.2 mg/min for body. Inhalation is considered negligible because of the low volatility of the substance and the short duration of the exposure. Tier 2 is not necessary.

	Parameters	Value	Reference
Tier 1	Frequency of events	52 $year^{-1}$	TNsG (2002)
	Duration: *Fluid monitoring* (min/event)	10	RMS assumption
	Body weight (kg)	60	HEAd Hoc Recommendation no. 14
	Respiration volume (m^3/hour)	1.25	HEAd Hoc Recommendation no. 14
	Weight fraction of active substance (Bronopol)	10 ppm (0.001%)	Worst-case product Applicant's data.
	Dermal absorption	50%	Guidance on Dermal Absorption (EFSA. 2017)
	Potential hand exposure	35.8	Recommendation 4. 2014.
	Potential body exposure	19.2	Recommendation 4. 2014.
Tier 2	Gloves penetration factor	10%	HEEG Opinion 9
	Coated coverall penetration factor	20%	HEEG Opinion 9

Calculations for Scenario 3

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 3	1 / none	--	4.60E-05	4.60E-05
Scenario 3	2 / PPE	--	6.20E-06	6.20E-06

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m^3)**	**Estimated dermal concentration (ppm)**
Scenario 3	1 / none	--	10 ppm
Scenario 3	2 / PPE	--	10 ppm

Scenario 4 – Post application: process equipment maintenance and RO systems

Description of Scenario 4
During the equipment maintenance and reverse osmose (RO) systems. professionals may be exposed to the biocidal product. The closest model found in the BEAT database (2008) is 'Cleaning of spray equipment', which includes rinsing and rubbing (with paper, rag or brush) tasks, which are similar in cleaning dispensing pumps or fouled system. The indicative exposure values for dermal exposure are as follows: 35.8 mg/min for hands; 19.2 mg/min for body. Inhalation is considered negligible because of the low volatility of the substance and the short duration of the exposure. Duration and frequency guidance were not indicated in the TNsG for equipment maintenance. however it is anticipated that this task could occur 8 hours per day on a daily basis. Workers can be exposed to diluted biocidal products. *i.e.* up to 10 ppm a.s. at maximum Tier 2 is not necessary.

	Parameters	Value	Reference
Tier 1	Frequency of events (event/day)	1	RMS assumption
	Duration: Equipment maintenance (min/event)	480	Worst-case scenario (8h)
	Body weight (kg)	60	HEAd Hoc Recommendation no. 14. 2017
	Respiration volume (m3/hour)	1.25	HEAd Hoc Recommendation no. 14. 2017
	Concentration of a.s in preserved fluids	0.001%	Worst-case product
	Dermal absorption	50%	Guidance on Dermal Absorption (EFSA. 2017)
	Exposure indicative value of hands (mg/min)	35.8	Cleaning of spray equipment. BEAT
	Exposure indicative value of body (mg/min)	19.2	Cleaning of spray equipment. BEAT
Tier 2	Gloves penetration factor	10%	HEEG Opinion 9
	Coated coverall penetration factor	20%	HEEG Opinion 9

Calculations for Scenario 4

Systemic effects

Summary table: systemic exposure from professional uses				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario 4	1 / none	--	2.21E-03	2.21E-03
Scenario 4	2 / PPE	--	2.98E-04	2.98E-04

See calculation spreadsheets available in Appendix II.

Local effects

Summary table: local exposure from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg a.s./m^3)**	**Estimated dermal concentration (ppm)**
Scenario 4	1 / none	--	10 ppm
Scenario 4	2 / PPE	--	10 ppm

Scenario 5 – Primary Exposure - Post-application: disposal of waste

Description of Scenario 5
According to the TNsG (2002) for PT 12 (p.97). process water is either recycled or discharged to waste treatment via drainage systems. Drums may be returned or recycled. and IBC's are returned to the supply company. Due to the closed nature of these drainage systems. human exposure is not anticipated during this process. The highest potential for exposure when handling these drums would occur during the drum changeout operation when transferring the dip tube from one drum to another. This scenario is covered in detail in the mixing/loading scenario (see above). The potential exposure when handling empty concentrate drums is anticipated to be far less than those for the mixing/loading phase.

Combined scenarios

Post-application scenarios are combined as a worst case assumption. taking into account 8 hours as a worst-case for the duration of equipment maintenance:

Summary table: combined exposure				
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg a.s./kg/day)**	**Estimated dermal uptake (mg a.s./kg/day)**	**Estimated total uptake (mg a.s./kg/day)**
Scenario [2, 3]	Tier 1/no PPE	--	5.52E-01	**5.52E-01**
Scenario [2, 3]	2/ PPE	--	**3.73E-03**	3.73E-03

Scenario 6 – Secondary exposure to workers from papermills (vapour phase)

Exposure to humidified air containing residual biocide represents a potential secondary inhalation exposure scenario for workers. A very worst case scenario is developed to assess exposure to humidified air containing biocide.

To estimate secondary exposure to workers from papermills. it is seprated into different scenarios:

- Scenario [6a]: Inhalation exposure to vapour phase;
- Scenario [6b]: Inhalation exposure to aerosol phase;
- Scenario [6c]: Dermal exposure to aerosols.

Description of Scenario 6a
Regarding the high solubility of Bronopol (>300 g/L). the vapour pressure (0.0051 Pa at 20°C) and the very low concentration in water (up to 10 ppm (*i.e.* mg/L) in usual conditions). the volatility of Bronopol is considered extremely low. In other words. the quantity of substance that can transfer from water to air is extremely low. As a a very worst-case approximation. the Henry's law can be used to approximately estimate the partition of the substance between the atmosphere and the aqueous phase in equilibrium: $$\frac{Cair}{Cwater} = \frac{\mathrm{kH}}{\mathrm{RT}}$$ Where: kH: Henry's law constant $1.16*10^{-6}$ $Pa.m^3.mol^{-1}$ RT: = 2436 $Pa.m^3.mol^{-1}$ (from the ideal gas constant and at 293K) C_{water}: 10 mg/L

	Parameters	Value	Reference
Tier 1	C_{air} (mg/m^3)	4.76E-06	Calculated value
	Body weight (kg)	60	HEAd hoc Recommendation no. 14
	Respiration volume (m^3/day) (8-hour)	16	HEAd hoc Recommendation no. 14

Calculations for Scenario 6a

Systemic effects

Summary table: estimated exposure from professional uses					
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg AS/kg bw/d)**	**Estimated dermal uptake (mg/kg bw/d)**	**Estimated oral uptake (mg AS/kg bw/d)**	**Estimated total uptake (mg/kg bw/d)**
Scenario [6a]	1/no PPE	1.27E-06	-	-	1.27E-06

Local effects

Summary table: estimated exposure concentration from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg pure BNP /m^3)**	**Estimated dermal concentration (ppm pure BNP)**
Scenario [6a]	1/no PPE	Negligible (1.27E-06)	n.r.

Scenario 6b – Secondary exposure to workers from papermills (aerosol phase)

Description of Scenario 6b
Inhalation exposure - Aerosol phases There is no model for assessing exposure to the aerosol part of the papermill system. As a worst case approach. the exposure to the aerosol of such systems is supposed to be lower than the application of liquid by spraying (worst-case value of TNsG from the spraying model 1 page 145: 405 mg product /m^3).

	Parameters	Value	Reference
Tier 1	Frequency of events (/day)	1	Worst-case
	Duration (hour)	8	Worst-case
	Concentration of Bronopol	10 ppm (0.001%)	Applicant's data
	Respiration volume (m^3/hour)	1.25	Recommendation no. 14. 2017
	Exposure indicative value (mg water/m^3)	405	Spraying model 1 (TNsG. 2002)

Calculations for Scenario 6b

Systemic effects

Summary table: estimated exposure from professional uses					
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg AS/kg bw/d)**	**Estimated dermal uptake (mg/kg bw/d)**	**Estimated oral uptake (mg AS/kg bw/d)**	**Estimated total uptake (mg/kg bw/d)**
Scenario [6b]	1/no PPE	6.76E-04	-	-	6.76E-04

Local effects

Summary table: estimated exposure concentration from professional uses			
Exposure scenario	**Tier/PPE**	**Estimated inhalation concentration (mg pure BNP /m^3)**	**Estimated dermal concentration (ppm pure BNP)**
Scenario [6b]	1/no PPE	4.05E-03	n.r.

Scenario 6c – Secondary exposure to workers from papermills (aerosol phase)

Description of Scenario 6c

Dermal exposure - Aerosol phases

Exposure to humidified air containing residual biocide represents also a secondary exposure for dermal contact with the aerosol phase of the air.

A reverse scenario approach is made in accordance with the CAR.

The Margin of Exposure for dermal exposure has been calculated by subtracting from the chronic systemic AEL of 0.08 mg ai/kg bw/d. the value of the total combined exposure of 6.77*10-4 mg/kg bw/d.

Margin of Exposure for dermal exposure	6.77*10-4	mg ai/kg bw/d
Exposure for one worker	0.081	mg ai/d
Exposure for product	8124	mg
Equivalent in kg or L	0.0081	kg

Then it appeared that 0.0081 kg of the product would be necessary for a worker to generate systemic effects due to the dermal secondary exposure. This dermal exposure can be then considered as very unrealistic.

Scenario 7 – Secondary exposure to workers from papermills (contact with paper)

Description of Scenario 7
Professionnals are in contact with the paper treated with the biocidal product. The following factors from ESD (European Commission. 2003)[16]. are taken into account to determine the remaining dose of Bronopol in paper considering 10 ppm Bronopol:

Loss with waste water during paper production	90%
Fraction of active ingredient remaining in paper	10%
Paper mill waste water (m^3/d)	5000
Daily paper production per mill (t/d)	333.3
Dry paper weight (g/m^2)	300

The following assumptions are made:

$$P = \frac{\frac{W}{90\%} \times 10\%}{D}$$

Where:

W: amount of active substance lost by water (kg/d)

D: daily paper production (kg/d)

P: amount of Bronopol in kg of dry paper (mg Bronopol/kg paper)

$$P = \frac{\frac{5000 \times 10}{90\%} \times 10\%}{3.33 \times 10^5}$$

It has been determined that the remainning dose of Bronopol in paper will be 16.7 mg/kg paper.

According to the intended uses Bronopol, the maximum concentration in process water is 10 ppm ai.

This value is equivalent to 5.001 mg ai/m^2 paper since the average weight of paper is 300 g/m^2.

A transfer coefficient of 30% was chosen based on transfer coefficients for dislodgeable residues reported in the TNsG Human exposure 2002 part 2[17].

	Parameters	Value	Reference
Tier 1	Residues of BNP in paper (mg/cm^2)	55 x 10^{-4}	Factors from ESD (EC. 2003)
	Exposed area of skin (cm^2) (½ surface area of hands)	420	HEAd Recommendation no. 14
	Transfer coefficient from paper to skin (worst-case)	30 %	TNsG 2002
	Dermal absorption of BNP	50%	Guidance EFSA
	Exposure frequency (10 per hour x 8 hours/day)	80/day	RMS assumption
	Body weight (kg)	60	HEAd hoc Recommendation no. 14

Calculations for Scenario [7]

Systemic effects

Summary table: estimated exposure from professional uses					
Exposure scenario	**Tier/PPE**	**Estimated inhalation uptake (mg/kg bw/d)**	**Estimated dermal uptake (mg/kg bw/d)**	**Estimated oral uptake (mg/kg bw/d)**	**Estimated total uptake (mg/kg bw/d)**
Scenario [7]	1/no PPE	-	4.20E-02	-	4.20E-02

Local effects

Local dermal exposure concentration is very difficult to assess since the BNP is incorporated in the

paper and it not possible to determine the remaining concentration after drying.

B.3.3.5. Non-Professional exposure

Non-professional uses are not foreseen for PT12.

B.3.3.6. Secondary exposure of the general public excluding dietary exposure

No exposure is foreseen for general public for PT12.

B.3.3.7. Dietary exposure

The following table summarises the intended use categories, their potential dietary exposure and the dietary exposure scenario associated.

Intended use category	Potential dietary exposure	Dietary exposure scenario
PT 12 - Slimicide treatment in the de-inking process of the pulp and paper.	Yes	Scenario FCM*
PT 12 - Slimicide treatment in the wet-end stage of the paper manufacturing process	Yes	Scenario FCM
PT 12- Online and cleaning in place preservation (biofouling control) of industrial RO /NF membranes	No	Not necessary (1)

* Food Contact Material
(1) Indirect exposure via food is not relevant for this intended uses as only industrial water and not potable water is concerned.

List of scenarios

Summary table of main representative dietary exposure scenarios			
Scenario number	**Type of use[1]**	**Description of scenario**	**Subject of exposure[2]**
1a.	Professional use	Slimicide treatment in the de-inking process of the pulp and paper and in the wet-end stage of the paper manufacturing process (PT 12)	Food
1b	Professional use	Slimicide treatment in the de-inking process of the pulp and paper and in the wet-end stage of the paper manufacturing process (PT 12)	Feed

[1] *e.g.* animal husbandry. food industry. professional use. residential use.
[2] *e.g.* chicken. milk. beer

Information of non-biocidal use of the active substance

Summary table of other (non-biocidal) uses			
	Sector of use	**Intended use**	**Reference value(s)**
1.	Veterinary use	- Control fungal infections in fin fish	No MRL established[1].
2.	Human use	- Excipient	No threshold established[2].
3.	Plant protection products	- Fungicide - Bactericide	Not Approval as active substance in accordance with Council Directive 91/414/EEC[3].

[16] European Commission; TGD on risk assessment, Chapter 2 and 7, 2003
[17] See transfer coefficients - dislodgeable residues from TNsG 2002 (part 2, p 204). See CAR C(M)IT/MIT, IIB PT 12, 2015 for further details

Summary table of other (non-biocidal) uses			
	Sector of use	**Intended use**	**Reference value(s)**
			Default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg 396 / 2005.
4.	Cosmetic products	- Preservative	Maximum concentration of 0.1 % of BNP to avoid formation of nitrosamines[4].

[1] EMEA/MRL/ 791/01-FINAL June 2001 COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS – BRONOPOL. COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010R0037-20230323).

[2] 22 November 2019 EMA/CHMP/302620/2017 Rev. 2* (*Rev. 2 includes an update of boric acid) - Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (SANTE-2017-11668) [https://www.ema.europa.eu/en/annex-european-commission-guideline-excipients-labelling-package-leaflet-medicinal-products-human].

[3] Commission Regulation (EC) No 2076/2002 of 20 November 2002 extending the time period referred to in Article 8(2) of Council Directive 91/414/EEC and concerning the non-inclusion of certain active substances in Annex I to that Directive and the withdrawal of authorisations for plant protection products containing these substances [https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32002R2076].

[4] REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 November 2009 on cosmetic products (recast) [https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1223]

Estimating transfer of biocidal active substances into foods as a result of professional and/or industrial application(s)

Bronopol degrades very quickly in paper processing at high temperature and the main metabolite that could remain in the aqueous medium that would be toxic is TNM, which is very soluble, it would go to water, it would not remain in the paper in the final processing of the paper in case there could be any residue left.

Scenario [1a] – Food packaging for PT 12

The intended use of BNP as slimicide treatment in the de-inking process of the pulp and paper and in the wet-end stage of the paper manufacturing process (PT 12) may result in food contamination via residues in paper used for food packaging. As detailed in the CAR of the active substance C(M)IT/MIT[18], a theoretical exposure has been calculated by the eCA for this estimation and is presented below:

As a first tier approach, the exposure to BNP via paper used for food packaging can be estimated using the worst case scenario of ESD (EC. 2003)[19]:

- Loss with waste water during paper production: 90%
- Fraction of active ingredient remaining in paper: 10%
- Paper mill waste water: 5000 m^3/d
- Waste water produced per ton dry paper: 15 m^3
- Daily paper production per mill: 200 t/d
- Dry paper weight: 300 g/m^2
- Packaging surface in contact with 1 kg food: 600 cm^2

According to the intended uses BNP concentration in water is 10 ppm. As a worst case, calculations were performed with 10 mg a.i./L (=10 g a.i./m3).
Then,

Amount of active substance lost by water:
W = 5000 m^3/d x 10 g ai/m^3 = 50 kg/d
Amount of active ingredient in the system per day:

[18] Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products - *Evaluation of active substances* - Assessment Report - C(M)IT/MIT - Product-type 12 (Biocide for use as slimcides) - April 2015 -France

[19] EC. 2003. Supplement to the methodology for risk evaluation of biocides. Harmonisation of Environmental Emission Scenarios for slimicides (product type 12). European Commission DG ENV/RIVM, September 2003. http://ecb.jrc.ec.europa.eu/documents/Biocides/EMISSION_SCENARIO_DOCUMENTS/ESD_PER_PRODUCT_TYPE/PT_12/PT_12_Slimicides.pdf

K = 50/100% = 50 kg/d

Amount of active substance remaining in dry paper:
A = 50 x 10%= 5 kg/d

It should be noted that the 10% factor is a worst case and takes into account the active ingredient remaining in dry paper and active ingredient loss in dry air.

Amount of active substance in kg of dry paper:
P = A/daily paper production = $5/(200x10^3)$ = 25 mg a.i./kg paper

Assuming a dry paper weight of 300 g paper/m^2, a conservative assumption of 1 kg food wrapped in 0.06 m^2 paper and 100% of residues migrate into the food and is consumed daily with a body weight of 60 kg. exposure would be:

Exposure = 25 x 10^{-3} mg a.i./g paper x 300 g paper/m^2 x 0.06 m^2 / 60 kg =
7.5 x 10^{-3} mg a.i./kg bw/d

Amount of a.s in dm^2 of paper: dry paper contains x dry paper weight = (25 mg a.i./kg paper) x (3 g/dm^2) = 0.075 mg a.s/dm^2

1 kg food wrapped in 0.06 m^2 paper (0.167 kg/dm^2), therefore**, the amount of active substance per kg of food is**: Amount of a.s in dm^2 of paper / 0.167 kg/dm^2 = **0.45 mg as/kg food**.

Default **MRL of 0.01 mg/kg** according to Art 18(1)(b) Reg 396 / 2005

Furthermore, a biocide intended to be used as a slimicide in process water for paper manufacturing may fall under the Food Contact Material Legislation. The maximum residual amount of BNP in food contact paper has not been derived by EFSA but the default value for FCMs data included in the corresponding regulation[20] is the default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg 396 / 2005.

Estimated residues in food from the intended use were 0.45 mg a.s./kg food (see AR B.3.3.7, p. 535), thereby exceeding the default MRL of 0.01 mg/kg that applies for bronopol (according to Art 18(1)(b) Reg 396 / 2005).

Therefore, for an adult, calculated daily exposure value of **7.5 x 10^{-3} mg a.i./kg bw/day** will be used to characterize risk from this indirect BNP exposure source. It should be noted that for children, no exposure scenario is available.

Conclusion
The daily exposure to BNP from food contamination via residues in paper used for food packaging was estimated to be **7.5 x 10^{-3}** mg a.i./kg bw/d for an adult.

Estimating transfer of biocidal active substances into foods as a result of non-professional use

No further transfer of the biocidal active substance into foods is anticipated.

Estimating Livestock Exposure to Active Substances used in Biocidal Products

Scenario [1b] – Feed packaging for PT 12

The intended use of BNP as slimicide treatment in the de-inking process of the pulp and paper and in the wet-end stage of the paper manufacturing process (PT 12) may result in food contamination via residues in paper used for food packaging. As feed may also be packaged with paper, livestock exposures have to be assessed.

This assessment is presented below:

[20] An official guideline is available in Germany for the use of different substances (e.g. slimicides) in the paper industry for paper and board that comes in contact with foodstuffs. It recommends that If slimicides and preservatives are used that have limit values according to (EG) Nr. 396/2005, these values are also valid for the migration from paper (see BfR Recommendation on Food Contact Materials, XXXVI. Paper and board for food contact, VII, b, 1, 2015; *http://www.bfr.bund.de/en/database_ bfr_recommendations_on_food_contact_materials_formerly__plastics_recommendations__-1711.html*).

Livestock exposures have been calculated using default scenario from the European Guidance document[21].

Results from *Scenario 1a – Food packaging* have been used for amount of active substance level in the paper (25 mg ai/kg paper). Assuming, as for human, that 1 kg feed is wrapped in 0.06 m² paper and 100% of residues migrate into feed, exposures are calculated as follows for each animal.

Exposure = quantity of as in paper for 1 kg feed x feed consumption per day / animal body weight

As proposed in the European Guidance document[15], screening is performed assuming that all of the feed the animal consumes comes packaged in treated paper/cardboard. Then, a worst-case estimation is performed assuming that 10% of feed is packaged in treated paper/cardboard. Density is assuming to be 300 g/m² for both food and feed packaging. Exposure results are presented in the table 116.

Table 116: Exposure results for livestock

	Animal body weight (kg)	**Animal feed consumption (kg/d)**	**Exposure from paper packaging (mg as/kg bw/d)**	
			Screening (100% wrapped feed/d)	**Worst-case (10% wrapped feed/d)**
Beef cattle	500	20	0.018	0.0018
Dairy cattle	650	26	0.018	0.0018
Laying hen	1.9	0.13	0.031	0.0031
Pig	100	3	0.0135	0.00135
Broiler	1.7	0.12	0.032	0.0032

Values: values > 0.004 mg as/kg bw/d

No exposure values calculated with the worst-case scenario (10% feed consumed wrapped) are above the trigger value of 0.004 mg a.i./kg bw/d. **It can therefore be concluded that no significant residues of active substance could occur in food of animal origin.**

B.3.3.8. Exposure associated with production, formulation and disposal of the biocidal product

Production and formulation are addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

B.3.3.9. Combined residential scenarios

None anticipated

[21] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

[21] Guidance on the Biocidal Products Regulation -Volume III Human Health - Assessment & Evaluation (Parts B+C) -6. Guidance on Estimating Livestock exposure to active substances used in Biocidal Product

B.4 Environmental exposure assessment

General information for PT 2 Scenarios

Assessed PT	PT 2
Assessed scenarios	Scenario 1: Emission scenario for disinfectants used in disinfection of chemical toilets (ESD JRC 2011, § 2.4, p.27)
ESD(s) used	Emission Scenario Document for Product Type 2: Private and public health area disinfectants and other biocidal products (sanitary and medical sector), JRC, 2011, Table 7, page 29); ECHA Excel template "Environmental Emission Scenarios for Product Type 2: Private and public health area disinfectants and other biocidal products" (v1.5 of January 2022).
Approach	Scenario 1: Average consumption
Distribution in the environment	Calculated based on TGD 2003 (alternative: based on measured data)
Groundwater simulation	Modelling with pearl 4.4.4.
Confidential Annexes	No confidential annex for the PT 2 scenarios required.
Lifecycle steps assessed	Scenario [1]: Production: No Formulation No Use: Yes Service life: Yes
Remarks	

General information for PT 11 Scenarios

TAB ENV 202, the refinement options for PT 11, indicates that the STP connection and treatment of cooling water before release to surface water should be only considered for small recirculating cooling systems. Hence, as the applicants state that all uses emissions will go through STP, hence the large cooling systems are not included.

Assessed PT	PT 11
Assessed scenarios	Scenario 2: Small open recirculating systems - Continuous Dosing - (ESD Table 7, p. 28). Scenario 3: Small open recirculating systems - Shock Dosing (ESD § 3.1.4.1). Scenario 4: Closed recirculating cooling systems -Shock and continuous Dosing (ESD Table 9, p.31).
ESD(s) used	Emission Scenario Document for Product Type 11: "Supplement to the methodology for risk evaluation of biocides. Harmonisation of environmental emission scenarios for biocides used as preservatives for liquid cooling systems (product type 11). European Commission DG ENV / RIVM, EUBEES, 2003"
Approach	Scenario 2: Average consumption Scenario 3: Average consumption Scenario 4: Average consumption
Distribution in the environment	Calculated based on TGD 2003 (alternative: based on measured data)
Groundwater simulation	Modelling with pearl 4.4.4
Confidential Annexes	No confidential annex for the PT 11 scenarios required.
Lifecycle steps assessed	Scenario 2: Production: No, Formulation: No, Use: Yes, Service life: No Scenario 3: Production: No, Formulation: No, Use: Yes, Service life: No Scenario 4: Production: No, Formulation: No, Use: Yes, Service life: Yes
Remarks	

General information for PT 12 Scenarios

Assessed PT	PT 12
Assessed scenarios	Scenario 5: Slimicide in paper industry, Typical case, STP Scenario 6: Slimicide in paper industry, Worst case, with STP Scenario 7: Worst case without STP Scenario 8: small factories exempted from Industrial Emissions Directive 2010/75/UE releasing to municipal STP, typical case Scenario 9: small factories, worst case
ESD(s) used	Emission Scenario Document for Product Type 12 - Harmonisation of Environmental Emission Scenarios Biocides: PT 12 – Slimicides. Royal Haskoning, NL, September 2003
Approach	All scenarios: Average consumption
Distribution in the environment	Calculated based on TGD 2003 (alternative: based on measured data)
Groundwater simulation	Modelling with FOCUS pearl 4.4.4.
Confidential Annexes	No confidential annex for the PT 12 scenarios required.
Lifecycle steps assessed	Production: No, Formulation: No, Use: Yes, Service life: Yes
Remarks	

The biocidal information intended for PT 12 are used in the wet end of paper mills to control the growth of target organisms in the circulating process water used in these systems. Emission was estimated for the realistic worst-case scenario and typical case scenario according to the ESD.

Further, and considering the last WG decisions on PT12, the worst-case scenario has to be divided in worst case, without STP (according to ESD) and worst case, with STP (according to the Industrial Emission Directive and the respective BAT). Because the IED and BAT do not restrict the operating procedure of large paper factories, such factories could operate according to the typical or to the realisitc worst case concerning the biocidal treatment of the long or short circulation water of a paper mill and concerning the connection to a pulp mill with slimicide-free wastewater.

Hence, paper factories that produce >20 tonne/d must comply to the Best Available Techniques (BAT) for the production of pulp, paper, and board, which basically demands a waste water treatment for large paper factories. For further details, please refer to:

Commission Implementing Decision of 26 September 2014 establishing the best available techniques (BAT) conclusions, under Directive 2010/75/EU of the European Parliament and of the Council, for the production of pulp, paper and board (2014/687/EU).

Available techniques consist of primary and secondary treatment. The first step concerns physico-chemical treatment in order to reduce the load for the second step, the second biological treatment including secondary sedimentation to remove sewage sludge.

The respective details are described in following document:

Best Available Techniques (BAT). Reference Document for the Production of Pulp, Paper and Board. Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control), Publications Office of the European Union EUR 27235 EN, Luxembourg, 2015.

It is therefore unimaginable that large paper factories release their waste water without sophisticated waste water treatment as suggested in the realistic worst-case scenario. For the risk assessment primary and secondary sedimentation and aeration is therefore considered for both scenarios, *i.e.* assuming waste water treatment that is in accordance with SimpleTreat. Consequently, a risk mitigation measure stating that wastewater must be treated in accordance with the BAT should be added for large paper factories. The factory's wastewater must be purified on-site in a sewage treatment plant as described in the Industrial Emission Directive 2010/75/EU (Best Available Techniques for the production of pulp, paper and board). A dilution of at least 200 times from the on-site WWTP to surface water must be achieved.

According to BPC-48 last decisions, "Application is only allowed in paper factories that comply with the Industrial Emission Directive 2010/75/EU where wastewater is purified in an on-site industrial

sewage treatment plant including a biological treatment step in accordance to the Best Available Techniques (BAT) as prescribed in the BAT-reference document (BREF) for the production of pulp, paper and board". Hence the typical case scenario with full on-site STP would be the only realistic case. Nevertheless, the worst case scenario has been included in the calculations as it is stated in the ESD for PT12. Paper factories discharge to surface water with different flow rate than the default Vol. IV part B&C river and paper factories produce more than 2000 m³ waste water daily. Therefore, concentrations in surface water were based on dilution factors as agreed for PT 11 *e.g.* 10 times for the default river (0.5 m³/s), 200 times for a river with a flowrate of 15 m³/s, and 1000 for 100 m³/s. Emission was calculated for all scenarios by using the default parameters.

Emission to soils and groundwater have been included, as agreed during ENV WGIII2023, although it is highly unlikely that sewage sludge that mainly consist in paper fibres will be applied as a soil fertiliser.

Paper factories that are not included in 2010/75/EU may discharge their wastewater to the municipal sewer (production capacity not exceeding 20 tonnes per day). In this case the soil compartment must be assessed due to possible municipal sludge application to soil.

B.4.1 Emission estimation

An environmental exposure assessment has been conducted based on the fate and distribution properties of the active substance, Bronopol, as determined from laboratory studies. Where appropriate the predicted environmental concentration (PEC) of substance Bronopol has been estimated in various environmental compartments (surface water, groundwater and air) following realistic worst case and, where appropriate, normal case usage scenarios for the formulated product.

As mentioned in section 4.2, hydrolysis products may be formed during the processing in cooling towers and paper pulp process. The hydrolysis is taking place in any process before the STP, but as there is no direct emission to surface water, it will always go through the STP, and the degradation in the STP is very high for 2-BNE (DT50 = 0.0047 h), hence, it is very unlikely that 2-BNE reaches surface water. The WGIV2022 decided that 2-BNE is covered by Bronopol only when emission is through STP, where 2-BNE is very rapidly degraded. In case of any direct emission to surface water, for a product authorisation, 2-BNE should be assessed.

Emission Assessment for Product Type 2

<u>Scenario 1:</u> Emission scenario for calculating the release of disinfectants used in disinfection of chemical toilets

The emission calculations were performed according to the Emission Scenario Document (ESD) for Product Type 2: "Private and public health area disinfectants and other biocidal products (sanitary and medical sector)", JRC, 2011.

Input	Symbol	Value	Unit	Remarks
Scenario 1: Release of disinfectants used in disinfection of chemical toilets				
Amount of biocidal product per litre pre-charge liquid in a chemical toilet	Vform	0.0001	L/L	100 ppm as efficacious recommended concentration and dosage claim
Fraction of active substance in the biocidal product	Fform	1	-	The product is 99-100% Bronopol
Density of the biocidal product	RHO_{form}	1.1	kg/L	Measured value for Bronopol
Volume of pre-charge liquid in the tank of a chemical toilet	Vliquid	20	L	Default value
Average amount of sewage in one mobile toilet before discharge (including pre-charge liquid)	Vsewage	60	L	Default value
Volume of the tank wagon collecting and discharging the sewage of chemical toilets	Vtank	2000	L	Default value

Number of tank wagons discharging to one local STP (standard size for 10000 inhabitants)	Ntank	1	d-1	Default value
Fraction of substance disintegrated during or after application (before release to the sewage system)	F_{dis}	0	-	Default: 0
Fraction of disinfectant released into the influent of the STP	$F_{influent}$	1	-	Default: 1
Output				
Amount of active substance in one tank wagon	Qa.i.tank		kg	
Local emission to STP from one tank wagon	ElocalSTP	0.0733	kg/d	
Qa.i.tank = Vform * Fform * RHOform * Vliquid * (1-Fdis) * Finfluent * (Vtank/Vsewage) ElocalSTP = Qa.i.tank * Ntank				

The following input-parameter tables (for all intended PTs) have been completed according to the advice given in the CAR template: "Please note only the values which have been included as "Set values" in the emission scenario, default values which are under discussion or when it is possible to choose between different defaults values should be stated in the table."

Table 117: Input parameters for calculating the local emission

The scenario for PT 2 was calculated based on the Exel calculation sheet "Emission scenario for disinfectants used in disinfection of chemical toilets (ESD JRC 2011,§ 2.4, p.27)" as implemented in the ECHA Excel template "Environmental Emission Scenarios for Product Type 2: Private and public health area disinfectants and other biocidal products" (v1.5 of January 2022).

Resulting local emission to relevant environmental compartments for scenario 1

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Freshwater	Not relevant	No direct releases of Bronopol to these environmental compartments; indirect releases only possible after passing STP
Sediment	Not relevant	
Seawater	Not relevant	
Seawater sediment	Not relevant	
STP	**$Elocal_{STP}$ = 0.0733 kg/d**	Emission to STP is sole direct emission pathway
Air	Not relevant	Emission to air is not relevant
Soil	Not relevant	No direct release of Bronopol to these environmental compartments
Groundwater	Not relevant	

Emission Assessment for Product Type 11

The following assessment was performed in accordance with the EU EUBEES Emission Scenario Document (ESD) titled "Harmonisation of Environmental Emission Scenarios for Biocides used as Preservatives for Liquid Cooling Systems", published by European Commission DG ENV in September 2003. According to this document, liquid cooling systems comprise, open re-circulating cooling systems and closed re-circulating cooling systems, which differ in volume and operation conditions.

Strong reduction of emissions to soil (and ground water) is taken into account by assuming drift elimination (efficacy 99%) for all intended cooling water applications. The respective RMM can be specified as "Cooling towers shall be equipped with eliminators that reduce drift by at least 99%." Emission estimation has been calculated both with and without drift elimination.

According to TAB ENV 202, the refinement options for PT 11, the STP connection and treatment of cooling water before release to surface water should be only considered for small recirculating cooling systems. Hence, as the applicants state that all uses emissions will go through STP, the large cooling systems are not included in the CAR. For this active substance, only emission via STP has been considered. Only small cooling systems with a maximum blowdown of 2 m³/h has been considered in this risk assessment.

Scenario 2: Emission scenario for small open recirculating systems – Continuous Dosing
In accordance with the advice implemented in the ECHA CAR template, only the values which have been included as "Set values" in the emission scenario, default values which are under discussion or when it is possible to choose between different defaults values are stated in the table.

Table 118: Input parameters for calculating the local emission

Input	Symbol	Value (small system)	Unit	Remarks
Scenario 2: small open recirculating systems – Continuous Dosing				
Nominal target concentration of a.i. in cooling water	Cproc a.i.	5	g/m³	Based on dosage claim (efficacious dose from 5 ppm)
Dose of formulated product to system	DOSE	1.5	kg	Based on ESD for PT 11 (2003)
Fraction of a.i. in product	Fform	1	-	
Water volume in the system	Vsyst	300	m³	
Blowdown flow rate	Qbld	2	m³/h	
Recirculating cooling water flow rate	Qcirc	100	m³/h	
Dosing interval	Tint	24	h	Worst case of a single dose each day (24 h interval default value in the ESD)
Duration of dosing	tdose	0.25	h	
Number of cooling towers per Site	N	1	-	
Degradation rate constant	kdeg	0.1507	h^{-1}	Hydrolyse with DT50 = 35,28 min at 50 ºC, TAB ENV 182 for temperature conversion to 20 ºC
Degradation rate constant, metabolites	kdeg metabolites	0	h^{-1}	No degradation in cooling systems in case of metabolites
Fraction evaporated + drift	Fevap+drift	0.01	-	No value given for small systems in ESD
Drift + evap rate	Qdrift+evap	1	m³/h	
Overall rate constant for removal from the cooling system	Ksyst	0.1607	h^{-1}	EUSES 2.1, model calculations III, eq. 311
Fraction deposited to soil	Fdepos	0.00025	-	
Soil surface where deposition occurs	AREAdepos	75000	m^2	

Evaporation	Fevap	0.04	-	
Volume of cooling air	Vair	10000000	m^{-3}	
Dilution factor air	DILUTIONair	100	-	
Output				
Continuous Amount of a.i. released per day and per site to water	RELEASEtcont	0.0096	kg/d	Continuous dosing
Release to air	RELEASEair	0.005	kg/d	With degradation
Dose of a.i. deposited to soil	DOSEpres	6.64E-8	g/m^2h	With degradation

$RELEASE_t$=N*Cbld*Qbld*t*0.001
RELEASEair=Fevap+drift*Qcirc*Cbld*0.001*N
$DOSE_{pres}$=Fdepos*Qcirc*Cbld/AREAdepos*N
Cbld=Cproc/(1+Ksyst*HRT)
HRT=Vsyst/(Qbld+Qevap_drift) =100 h (EUSES 2.1, model calculations III, eq. 310)

Resulting local emission to relevant environmental compartments scenario 2

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Freshwater (Continuous dosing concentration of a.i. in blowdown water)	**Small system: $C_{blowdown}$ = 0.293 mg/L**	Environmental concentrations (mg/L). Degradation in system.
E local water	0.014 kg/d	
Sediment	Not relevant	No direct release of Bronopol to sediment.
Seawater	Not relevant	No release of Bronopol to marine compartments
Seawater sediment	Not relevant	
STP	Not relevant	Emission pathway via STP is not relevant for this scenario.
Air	Not relevant	Only very low amounts of Bronopol will be released into air due to PT 11 application
Soil	Not relevant	No direct release of Bronopol to these environmental compartments
Groundwater	Not relevant	

<u>Scenario 3:</u> Emission scenario small open recirculating systems – Shock Dosing

Please note only the values which have been included as "Set values" in the emission scenario, default values which are under discussion or when it is possible to choose between different defaults values should be stated in the table.

Table 119: Input parameters for calculating the local emission

Input	Symbol	Value (small system)	Unit	Remarks
Scenario 3: small open recirculating systems – Shock Dosing				
Nominal target concentration of a.i. in cooling water	Cproc a.i.	5	g/m^3	Based on dosage claim
Dose of formulated product to system	DOSE	1.5	kg	Based on ESD for PT 11 (2003)
Fraction of a.i. in product	Fform	1	-	
Water volume in the system	Vsyst	300	m^3	Default value
Blowdown flow rate	Qbld	2	m^3/h	Default value

Recirculating cooling water flow rate	Qcirc	100	m³/h	Default value
Dosing interval	Tint	24	h	Default value
Duration of dosing	tdose	0.25	h	
Number of cooling towers per Site	N	1		
Degradation rate constant	kdeg	0.1507	h^{-1}	Hydrolyse with DT50 = 35,28 min at 50 ºC
Degradation rate constant, metabolites	kdeg metabolites	0	h^{-1}	
Fraction evaporated + drift	Fevap+drift	0.01	-	
Drift + evap rate	Qdrift+evap	1	m³/h	Default value
Overall rate constant for removal from the cooling system	Ksyst	0.1607	-	K_{syst}=(Qbld+ Qdrift+Qevap)/V_{syst} + Kdeg
Fraction deposited to soil	Fdepos	0.00025	-	
Soil surface where deposition occurs	AREAdepos	75000	m^2	Acc to Pt11 note (2013), p.1 A1
Evaporation	Fevap	0.04	-	Default value
Volume of cooling air	Vair	10000000	m³	Default value
Dilution factor air	DILUTIONair	100		Default value
Output				
Continuous Amount of a.i. released per day and per site to water	RELEASEt	0.06	kg/d	Shock dosing
Release to air	RELEASEair	3.05E-02	kg/d	With degradation
Dose of a.i. deposited to soil	DOSEpres	4.23E-07	g/m^2h	With degradation
Shock dosing concentration of a.i. in blowdown water	Cbld($t_{0,1}$)	1.27		
$RELEASE_t$=- Cproc*Qbld*(($e^{-(Ksyst*t)}$-1)/$K_{syst)}$*0.001*N $RELEASE_{max}$= Cproc*Qbld/K_{syst}*0.001*N Frelw= Qbld/(Qbld+ Kdeg*$V_{syst)}$ $Cbld_t$=Cproc*(1-$e^{-(Ksyst)*t}$)/(Ksyst*T) (Euses model calculations III, eq. 314)				

Resulting local emission to relevant environmental compartments in scenario 3

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Freshwater (Shock dosing concentration of a.i. in blowdown water)	**Small system: $C_{blowdown}$ = 1.27 mg/L**	Environmental concentrations (mg/L)
E local water	0.061 kg/d	
Sediment	Not relevant	No direct release of Bronopol to sediment compartment
Seawater	Not relevant	No release of Bronopol to marine compartments
Seawater sediment	Not relevant	
STP	Not relevant	No release to STP.
Air	Not relevant	Only very low amounts of Bronopol will be released into air due to PT 11 application No relevant release of Bronopol to these environmental compartments

Soil	Not relevant	No direct release of Bronopol to these environmental compartments
Groundwater	Not relevant	

Scenario 4: Emission scenario for closed circulating cooling systems – Shock and Continuous Dosing

Please note only the values which have been included as "Set values" in the emission scenario, default values which are under discussion or when it is possible to choose between different defaults values should be stated in the table.

Table 120: Input parameters for calculating the local emission

Input	Symbol	Value	Unit	Remarks
Scenario 4: Closed recirculating cooling systems – Shock and Continuous Dosing				
Nominal target concentration of a.i. in cooling water	Cproc a.i.	5	g/m^3	Based on dosage claim
Volume of water in system	Vsyst	30	m^3	Default value
Blowdown flow rate	Qbld	0.0004	m^3/h	Default value
Fraction lost during dosing event	Floss dosing	0.005	-	Default value
Fraction lost due to design	Floss design	0.01	1/month	Default value
Fraction lost at complete drainage	Floss drain	1	-	Default value
Hydraulic retention time	HRT	75000	h	Default value
Degradation rate constant in system	kdeg	0.1507	h^{-1}	
Output				
Overall rate constant for removal from the cooling system	Ksyst	0.1507		
No degradation – Release during dosing event	RELEASEdosing	7.50E-04	kg a.s/event	
No degradation – Release in process due to design	RELEASEdesign	1.50E-03	kg a.s/h	
No degradation – Release at complete drainage	RELEASEdrainage	0.15	kg a.s/event	
Degradation – Release after one single dose	RELEASEt	1.29E-05	kg a.s/event	
Degradation – Release in process due to design	RELEASEmax	1.33E-05	kg a.s/h	
Degradation – Fraction released to water after infinite time	Frelw	8.84E-05	-	

$K_{syst} = Qbld/V_{syst} + Kdeg$
$RELEASE_{dosing} = F_{loss\ dosing} * V_{syst} * Cproc$
$RELEASE_{design} = F_{loss\ design} * V_{syst} * Cproc$
$RELEASE_{drainage} = F_{loss\ drainage} * V_{syst} * Cproc$
$RELEASE_t = Cproc * Qbld * (e^{-(Ksyst*1)} - 1)/K_{syst} * 0.001$
$RELEASE_{max} = Cproc * Qbld/K_{syst} * 0.001$
$Frelw = Qbld/(Qbld + Kdeg * V_{syst})$

Resulting local emission to relevant environmental compartments scenario 4

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Freshwater	Not relevant	No direct release of Bronopol to these environmental compartments, only indirectly after passing STP
Sediment	Not relevant	

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Seawater	Not relevant	No release of Bronopol to marine compartments
Seawater sediment	Not relevant	
STP (Release after one single dose)	**1.29E-05 kg a.s./event 1.05E-01 kg a.s./event (worst case, drainage)**	Assumption: With degradation and worst case drainage without degradation
Air	Not relevant	No direct releases of Bronopol to these environmental compartments
Soil	Not relevant	
Groundwater	Not relevant	

Emission Assessment for Product Type 12

Scenario 5, 6, 7, 8, 9: Emission scenario for Slimicide Use in Paper Industry

The emission assessment was performed according to the ESD for Product Type 12 – "Harmonisation of Environmental Emission Scenarios Biocides: PT 12 – Slimicides. Royal Haskoning, NL, September 2003".

Further, TAB ENV 190, 203 and 238 have been considered.

Table 121: Input parameters for calculating the local emission

Input	Value					Unit	Remarks
	Sc 7: Realistic worst case (release without STP)	Sc 5: Typical case (release via STP)	Sc 6: worst case (with STP)	Sc 8: small factories typical case	Sc 9: small factories worst case		
Scenario 5 – 9: Slimicide use in paper industry							
Concentration of use in the system (continuous concentration), Cprod	10	10	10	10	10	g/m^3	Dosage claim
Rate constant for hydrolysis	3.62 / 14.89	14.89	3.62 / 14.89	14.89	3.62 / 14.89	1/d	Kdeg at 20 °C (TAB ENV 190) and at 40 ºC for the white water circuit (WGII2023).
Time elapsed since dosing event	1	1	1	1	1	d	
Fraction of the biocide lost in the dry end of the papermaking machine, Ftotal loss	0.1	0.1	0.1	0.1	0.1		Default values
Fraction of the total wastewater flow coming from the short circulation of the wire part, Fww1	1	0.6	1	0.6	1		Default values.
Fraction dilution wastewater with wastewater from pulping, Fww2	0	0.5	0	0.5	0		Default values

Input	Value					Unit	Remarks
	Sc 7: Realistic worst case (release without STP)	Sc 5: Typical case (release via STP)	Sc 6: worst case (with STP)	Sc 8: small factories typical case	Sc 9: small factories worst case		
Hydraulic retention time for paper making process, Tpr	0.167	0.167	0.167	0.167	0.167	d	
Fraction of biocide adsorbed to particles during primary settling, Fads, settling	0	0	0	0	0		
Output							
Theoretical concentration of a.i. in paper mill, Cpaper*	9.00	2.70	9.00	2.70	9.00	g/m^3	calculation from ESD PT 12 p.9
Degradation rate during paper prod. Process =degradation in paper mill + Hydrolysis	3.62 / 14.89	14.89	3.62 / 14.89	14.89	3.62 / 14.89	1/d	Only hydrolysis has been considered (TAB ENV 238)[a]
Semi-continous Dosing – concentration in influent to the primary settler, Cinf, ps	3.79	0.77	3.79	0.77	3.79	mg/L	ESD PT 12 (2003) Calculations for [A] (page viii) (semi)continuous dosing
Concentration in effluent of the water treatment system, Clocacleffl, treat	1.13	-				mg/L	ESD PT 12 (2003) (page viii)
Concentration in influent to STP, ClocalinfSTP		0.77	3.79	0.77	3.79	mg/L	TAB ENV 203**
Elocal water		3.87	18.96	0.23	1.14	kg/d	For an on-site STP (industrial STP, 5000 m3 discharge rate); municipal STP, 300 m3 (15 m3 of wastewater/ton * 20 tn/day)

*Cpaper = Cprod * Fww1 * (1-Fww2) * (1-Ftotal loss)

**TAB ENV 203:

Clocal eff treat = Cinf-ps * (1-Fads,settling – F ads,cm)*exp(-k deg2 (Tps+Tcm))

ClocalinfSTP = Cpaper * (1-Fads,settling) * exp (-kdeg1 (Tpr+Tps))

kdeg1 for hydrolysis (depending on T)

kdeg2 for hydrolysis plus biodegradation (in this case there is no surface water degradation test, hence only hydrolysis is considered, TAB ENV 238: only abiotic degradation taken into account in the industrial process, unless reliable further studies justify additional degradation).

[a]As discussed during ENV WG II 2023, a temperature of 40 ºC would be applicable for the primary circuit in the paper industry (the WG agreed to apply 40°C for the white water circuit, but 20°C for the remaining part of the machine, WG-II-2023, item 7.3); due to this higher temperature than the default of 20 ºC from TAB ENV 190, Bronopol hydrolysis will be much favoured in the white water circuit in the typical case scenario and partially in the worst case scenario. In order to achieve this, two cautions must be made for the worst-case scenario *i.e.* hydrolysis at 40°C and lowering Fww1 to 0.6 (equal to the typical case scenario that only considers the white water circuit), and hydrolysis at 20°C and Fww1 set to 0.4. The concentrations are subsequently summed up to derive the PECs for the worst-case. The typical case scenario only should consider the white water circuit at 40°.

PECstp is derived by multiplying the concentration before primary settling (Cinfls, ps) with the fraction in the STP as derived with SimpleTreat 4.

Regarding the small paper factories (< 20 tn/d) releasing to municipal STP, the dilution factor from the municipal STP to the surface water is limited to 10 according to BPR guidance vol IV part B+C, section 2.2, which states that "A fixed dilution factor of 10 is applied to the effluent concentration of an STP (by default assumed to be present)".

In the realistic worst-case, after a primary on-site treatment, the effluent would be released onto the surface water. This is not considered realistic, as mentioned before, as most of the paper mills has an on-site secondary/biological treatment. Nevertheless, the calculations have been included for completeness.

Resulting local emission to relevant environmental compartments in typical case scenario and worst case with STP

Compartment	Local emission ($Elocal_{compartment}$) [kg/d]	Remarks
Freshwater	Not relevant	No direct releases of Bronopol to these environmental compartments, only indirectly after passing STP
Sediment	Not relevant	
Seawater	Not relevant	No release of Bronopol to marine compartments
Seawater sediment	Not relevant	
STP (Concentration in influent to STP)	**$Clocal_{infl\text{-}STP}$ = 0.77 mg/L (typical case); 3.79 mg/L (worst case)**	Environmental concentration (mg/L). Emission to STP is sole direct emission pathway
Air	Not relevant	No relevant release
Soil		No direct releases of Bronopol to these environmental compartments. Nevertheless, sludge from the STP might be applied to the agricultural soil, hence PECsoil and PECgw should be included in the risk assessment. For those industrial facilities under the remit of the Industrial Emission Directive, although very unlikely, the sludge application to soil is not impossible, hence also PEC soil and gw are considered.
Groundwater		

For the worst-case scenario, rounded calculated dilution factors considering a 5000 m^3/d STP, and the TGD river flow rates of 0.5, 15 and 100 m^3/s are 10, 200 and 1000.

B.4.2 Fate and distribution in exposed environmental compartments

It is clear that, due to physical-chemical characteristics of the compound (weak affinity for sorption to organic carbon, biodegradable), that the majority of the load in wastewater will stay in the aqueous phase, with a component being degraded within the STP. In order to quantify this, estimates of distribution of the compound within STP compartments have been made using the SimpleTreat 4.0.

This model is a multi-compartment box model, calculating steady-state concentrations in a sewage treatment plant, consisting of a primary settler, an aeration tank and a liquid-solid separator. The use of this model is consistent with guidance presented in the Technical Guidance Document (European Commission, 2003). The model takes into account the Henrys law constant and the n-octanol water partitioning coefficient of the compound to predict partitioning behaviour. In the case of Bronopol, these values are $1.16*10^{-6}$Pa*m^3*mol^{-1} and logKow of -0.42, respectively. The selection of the appropriate fate characteristics is also dependent upon the biodegradability of the compound, as determined by a ready biodegradability or inherent biodegradability study. The fate and distribution characteristics for Bronopol in the standard STP plant described in the Technical Guidance Document (including primary settler) determined from the SimpleTreat 4.0 Model are summarised in the Table below.

The estimated distribution of Bronopol within STP determined on the basis of Henrys Law Constant and the n-octanol water partitioning coefficient using the SimpleTreat 4.0 Model and considering method 1 as only one sludge was used in the OECD TG 314B study:

Fate	% distribution
to air	0
to water	2.804
to sludge	1.22
degraded	95.97
total	100

Based upon the estimated distribution values presented in the table it can be seen that the majority of the Bronopol load (95.97%) will be degraded within the STP, with a 2.8% being directed to water and 1.22 % directed to sludge. The SimpleTreat calculations indicate that losses of Bronopol to air components of a STP are negligible. Therefore, no further consideration of this exposure pathway is necessary.

Table 122: Identification of relevant receiving compartments based on the exposure pathway

	Freshwater	Sediment	Seawater	Seawater sediment	STP	Air	Soil	Groundwater
PT2 Scenario 1: Chemical toilets	(Yes)	(Yes)	No	No	Yes	No	(Yes)	(Yes)
PT11 Scenario 2: Open systems – Small contin. dosing	(Yes)	(Yes)	No	No	Yes	Yes	(Yes)	(Yes)
Scenario 3: Open systems small shock dosing	(Yes)	(Yes)	No	No	Yes	Yes	(Yes)	(Yes)
Scenario 4: Closed systems with degradation	(Yes)	(Yes)	No	No	Yes	No	(Yes)	(Yes)

	Freshwater	Sediment	Seawater	Seawater sediment	STP	Air	Soil	Groundwater
PT12 Scenario 5-6: Slimicide typical and worst with STP	(Yes)	(Yes)	No	No	Yes	No	(Yes)	(Yes)
Scenario 7: Worst case as in ESD (no STP)	Yes	(Yes)	No	No	No	No	No	No
Scenario 8-9: Small factories	(Yes)	(Yes)	No	No	Yes	No	(Yes)	(Yes)

Indirect exposure pathway indicated in brackets

Table 123: Input parameters (only set values) for calculating the fate and distribution in the environment

Input	Value	Unit	Remarks
Molecular weight	**199.99**	g/mol	
Melting point	**129**	°C	
Boiling point	**-**	°C	
Vapour pressure (at X °C)	**0.0051**	Pa	**20 °C**
Water solubility (at X °C)	**304000**	mg/L	**20 °C**
Log10 Octanol/water partition coefficient	**-0.42**	---	
Organic carbon/water partition coefficient (Koc)	**136**	L/kg	
Henry's Law Constant (at 25 °C) [if measured data available]	**1.160E-06**	Pa * m^3/mol	
Biodegradability	**Not readily**		
Rate constant for STP [if measured data available]	**2.99**	h^{-1}	**Referred to 15°C**
DT50 for biodegradation in surface water	**-**	d or hr (at 12 ºC)	
DT50 for hydrolysis in surface water	**0.36**	d (at 12 ºC /pH)	**pH 7**
DT50 for photolysis in surface water	**20 days**	d or hr	
DT50 for degradation in soil	**300 d**	d or hr (at 12 ºC)	Worst-case default according to table 6 (ERA, 2017) considering: - Bronopol is a borderline case for readily biodegradability. - According to the supporting information regarding readily biodegradability (A7.1.1.2.1_01) bronopol was biotransformed by day 29 to 92 %. According to ECHA Guidance R7": "when results of ready degradability tests indicate that the pass level criterion is almost fulfilled, such results can be used as evidence for inherent biodegradability. - In the inherent test the parent substance disappears completely at day 3 (supporting information). - The half-life of Bronopol due to degradation

Input	Value	Unit	Remarks
			in natural waters (literature supporting information) is 0.03 h. If a DT50 for surface water is available, it may serve as basis for a DT50 in soil derived from table 6 as it is done with screening tests. - It has a very low Kp of 2.72 L/kg.
DT50 for degradation in air	**12.1 d**	d or hr	AOPv.1.91

Degradation products	TNM
Molecular weight	151.12
Log Octanol/water partition coefficient (LnowKow)	LnowKow (KOWWIN v1.68 estimate) = -1.66
Organic carbon/water partition coefficient (Koc)	10 L/kg (MCI method)
Henry's Law Constant (25 °C)	[HENRYWIN v3.20]: 4.88E-007 Pa-m^3/mole
Biodegradability	Not readily biodegradable (QSAR prediction: YES)
DT50 for biodegradation in surface water	n/a
DT50 for hydrolysis in surface water	3.42 d at 25 ºC (9.2 d at 12 ºC)
DT50 for photolysis in surface water	n.a.
DT50 for degradation in soil	n.a.
DT50 for degradation in air	n/a
DT50 for degradation in sediment	n/a
Degradation rate in the STP (CAKE)	0.01474 h^{-1} (15 ºC)

Table 124: Calculated fate and distribution in the STP [if STP is a relevant compartment]

Compartment	Percentage Bronopol [%]	Percentage TNM [%]	Remarks
Air	0	0	Calculated using SimpleTreat 4.0; used for all ENv RAs
Water	2.804	85.54	
Sludge	1.22	0	
Degraded in STP	95.97	14.46	

B.4.3 Calculated PEC values

PEC values have been estimated for both parent and metabolite TNM, as decided in WG-IV-2022, considering the only release pathway via the STP. The only direct release to surface water would be the "worst case scenario" for PT12, but as previously mentioned, the BAT document includes as available techniques primary and secondary treatment (typical case scenario), hence worst case scenario is very unlikely.

For transformation products, in this case, TNM as main metabolite to be assessed, TAB ENV 213 should be used. A conservative tier 1 assessment of TPs formed in the STP is that distribution and degradation processes are not considered in the assessment. Thus, only the fraction "degraded" of the parent run of SimpleTreat 4.0 is taken into account for the TP formed. Afterwards, it is assumed that 100% of each of the formed TPs is emitted via the STP effluent as well as via sewage sludge application. For TNM, simple treat estimations lead to no fraction released to sludge, hence only Clocal to water must be considered.

The relevant equation following the equation of the BPR guidance Part B+C (2017) with some minor additions, are:

Clocaleff_TP (analogous to eq. 36):

$$Clocal_{\text{eff\_TP}} = Clocal_{\text{inf\_Parent}} * F_{STP\_degraded} * f_{ij} * \frac{Mass_{molar_{TP}}}{Mass_{molar_{Parent}}}$$

fij (fraction of TNM actually formed from parent in the STP) = 99.75% (whole time from CAKE simulation). This value is derived from the analytical measurements in the 314B study. The DT50 for TNM in ths STP is 1.17 days at 20.55 ºC, which results in a kdeg in the STP at 15 ºC = 0.01474 h^{-1}.

A tier 2 refinement could be possible according to Annex 1 in TAB ENV 213, by simulating the TP in the STP; these are the results obtained in simple treat with the kdeg obtained in CAKE: TNM degrades 14.46% and the 85.84% partitions to surface water (0% to sludge).

$$Clocal_{\text{inf\_TP}} = Clocal_{\text{inf}}{}_{Parent} * F_{STP\_degraded} * f_{ij} * \frac{Mass_{molar_{TP}}}{Mass_{molar_{Parent}}} \qquad \text{Eq.4}$$

Regarding the possible mixture assessment of the parent and of the metabolite TNM, the mixture toxicity seems unlikely, because of the following:

- TNM may be formed mainly biotically in the STP, but it can also be formed in a chemical toilet or in the sewer where not only abiotic degradations such as hydrolysis will be taking place. In such cases, Bronopol rapidly and highly degrades to TNM and other degradation products. As all emissions are going through STP, OECD TG 314B should be considered as a basis for stating that Bronopol and its main degradation product, TNM are very unlikety to coexist. According to CAKE simulation based on OECD TG 314B, the transformation rate from Bronopol to TNM is 99.75%, summed over the whole time period of parent substance degradation:

Parameter	Initial Value	Bounds	Fixed
BN_0	90.96	0 to (unbounded)	No
k_BN	181.8	0 to (unbounded)	No
f_BN_to_TNM	0.9975	0 to 1	No
TNM_0	0	0 to (unbounded)	Yes
k_TNM	0.5666	0 to (unbounded)	No

- In case of PT2, the risk assessment has considered that all Bronopol is reaching the STP, hence, no TNM is being considered until the STP formation. In case of PT11 and 12, only abiotic degradation of the parent during the industrial process and according to the ESDs has been considered, hence no TNM is expected until the STP.

Table 125: Summary table on calculated PEC values for Bronopol

	PEC_{STP} [mg/L]	**PECwater** [mg/L]	**PECsed** [mg/kgdwt]	**PECsoil** (mg/kgwwt)	**PEC_{GW*}** [µg/L]	**PECair** [mg/m^3]
PT2						

	PEC_{STP} [mg/L]	**PECwater** [mg/L]	**PECsed** [mg/kgdwt]	**PECsoil** (mg/kgwwt)	**PEC_{GW*}** [µg/L]	**PECair** [mg/m^3]
Scenario 1: Chemical toilets	1.03E-04	1.03E-05	1.77E-04	2.39E-04	7.74E-02	n.a.
PT11						
Scenario 2: Open recirculating cooling systems – Continuous dosing	1.34E-04	1.34E-05	2.30E-04	3.16E-04	0.10	n.a
Scenario 3: Open recirculating cooling systems – Shock dosing	8.54E-04	8.54E-05	1.47E-03	1.99E-03	0.64	n.a
Scenario 4: Closed recirculating cooling systems – Continuous dosing a)Drainage (worst case) b)With degradation	a)2.10E-03 b)1.81E-07	a)2.10E-04 b)1.81E-08	a)3.62E-03 b)3.11E-07	a)4.89E-03 b)4.21E-07	a)1.58 b)1.36E-04	n.a
PT12						
Scenario 5: Slimicide Paper Industry Typical case	2.17E-02	1.08E-04	1.86E-03	5.99E-02	19.4	n.a
Scenario 6: worst case with STP	1.06E-01	5.32E-04	9.14E-03	2.93E-01	94.92	n.a.
Scenario 7: worst case direct release** (max DF of 1000)	n.a.	1.13E-03	1.95E-02	n.a.	n.a.	n.a.
Scenario 8: small factories typical case	2.17E-02	2.17E-03	3.73E-02	2.04E-02	6.59	n.a.
Scenario 9: small factories worst case	1.06E-01	1.06E-02	1.83E-01	2.71E-02	8.77	n.a.

*ECGW was refined by using a simulation tool (FOCUS pearl 4.4.4.), the results for the different simulated scenarios is provided below in a separate table.
**Worst case very unlikely due to BAT document including secondary treatment for paper mills > 20 ton/day. Further, from BPC, a RMM of a secondary treatment is required (full on-site STP).

Table 126: Summary table on calculated PEC values for TNM

TNM (no partition to sludge, hence no risk to soil or GW applicable*)	**PECwater** [mg/L]	**PECsed** [mg/kgdwt]
PT2 – Scenario 1: Chemical toilets	2.27E-03	1.04E-02
PT11		
Scenario 2: Open recirculating cooling systems – Continuous dosing	4.35E-04	2.00E-03

TNM (no partition to sludge, hence no risk to soil or GW applicable*)	PECwater [mg/L]	PECsed [mg/kgdwt]
Scenario 3: Open recirculating cooling systems – Shock dosing	1.88E-03	8.67E-03
Scenario 4: Closed recirculating cooling systems – Continuous dosing – Worst case total drainage (best ca–e - With degradation	4.64E-03 (3.99E-07)	2.13E-02 (1.84E-06)
PT12		
Scenario 5: Slimicide Paper Industry typical case scenario	2.40E-03	1.10E-02
Scenario 6: worst case with STP	1.17E-02	5.40E-02
Scenario 8: Small factories typical case	4.79E-02	2.20E-01

*The degradation product TNM is formed in the STP but it does not partition to sludge to soil. Nevertheless, a worst case has been considered: all bronopol released to the soil compartment from sludge application, once in soil, degrades to TNM with a formation fraction of 100%. By considering the OECD TG 307 pretest results and FOCUS refinement when needed, there is no risk to groundwater.

PEC in surface water, ground water and sediment

In all scenarios, emissions to surface water at the local scale were calculated from emissions to sewage and assuming the standard STP fed by 10,000 inhabitant equivalents, as given in the relevant guidance.

The emissions through STP consider the formation of TNM as main degradation product and hence this metabolite has been assessed.

PEC in air

Emission scenarios presented in the ESD for PT2, PT11 closed recirculating and PT12 predict no or negligible losses to air when a biocidal product is applied in the respective manner. This prediction of negligible/zero loss to air is supported by the predicted Henry's law constant of $1.16*10^{-6}$Pa.*m^3.mol^{-1}. Furthermore, SimpleTreat v4.0 modelling for the behavior of bronopol at local STP predicts that the fraction directed to air ($Fstp_{air}$) would represent only 6.89E-07 % of the quantity of compound arriving at the treatment plant in wastewater, corresponding to Cair of 1.86E-13. These assumptions are also supported by the environmental screening study conducted in Sweden in 2005. The aim of the screening was to determine concentrations of biocides in several media, including air. Bronopol was not found at any sampling location with a LOD of 0.05-0.07 ng.m^{-3}.

PEC in soil

It is considered that, due to the controlled manner in which Bronopol will be handled, there is no obvious intended primary exposure route to local soil. All emissions are predicted to reach the environment solely by washing or rinsing with discharge of wastewater to local STP. Therefore, no assessment of direct exposure from Bronopol via this route has been undertaken as Bronopol is not considered to be of concern for this environmental compartment.

There is one exception, emissions to soil from PT11 open cooling systems, where direct emission to soil is possible via drift. The results with and without drift elimination are included here. The drift elimination is needed for a safe use:

Small open continuous

Without drift elimination					
Dose of a.i. deposited to soil	g.m-2.h-1	DOSEpres	9,76E-08	O	with degradation
Dose of a.i. deposited to soil	g.m-2.d-1	DOSEpres	2,34E-06	O	with degradation
Depth of the receiving soil compartment	m	Depth	0,2	D	Default value based on BPR guidance vol IV, part B+C
Density of wet bulk soils	(kg/m3)	RHO	1700	D	Default value based on BPR guidance vol IV, part B+C
Soil-water partitioning coefficient	m3/m3	Ksoil-water	4,28	O	Default value based on BPR guidance vol IV, part B+C
first order rate constant for removal (12 ºC)	(d-1)	k	2,87E-03	D	Default value based on BPR guidance vol IV, part B+C
Csoil = DOSEpres/Depth*RHO*k-DOSEpres/ Depth*RHO*k*exp(-T*k)	mg/Kgwwtsoil	PECsoildrift	1,56E-03	O	According to TMIII2013
Predicted concentration in porewater	µg/L	PEC groundwater	0,62	O	

With 99% drift elimination					
Dose of a.i. deposited to soil	g.m-2.h-1	DOSEpres	9,76E-10	O	with degradation
Dose of a.i. deposited to soil	mg.m-2.d-1	DOSEpres	2,34E-08	O	with degradation
Depth of the receiving soil compartment	m	Depth	0,2	D	Default value based on BPR guidance vol IV, part B+C
Density of wet bulk soils	(kg/m3)	RHO	1700	D	Default value based on BPR guidance vol IV, part B+C
first order rate constant for removal (12 ºC)	(d-1)	k	2,87E-03	D	Default value based on BPR guidance vol IV, part B+C
Csoil = DOSEpres/Depth*RHO*k-DOSEpres/ Depth*RHO*k*exp(-T*k)	mg/Kgwwtsoil	PECsoildrift	1,56E-05	O	According to TMIII2013
RELEASEair	kg.d-1	RELEASEair	7,03E-05		
Pedicted concentration in porewater	µg/L	PECgroundwater	0,03	O	

Small open shock

Without drift elimination					
Dose of a.i. deposited to soil	g.m-2.h-1	DOSEpres	4,23E-07	O	with degradation
Dose of a.i. deposited to soil	g.m-2.d-1	DOSEpres	1,02E-05	O	with degradation
Depth of the receiving soil compartment	m	Depth	0,2	D	Default value based on BPR guidance vol IV, part B+C
Density of wet bulk soils	(kg/m3)	RHO	1700	D	Default value based on BPR guidance vol IV, part B+C
Soil-water partitioning coefficient	m3/m3	Ksoil-water	4,28	O	Default value based on BPR guidance vol IV, part B+C
first order rate constant for removal (12 ºC)	(d-1)	k	2,87E-03	D	Default value based on BPR guidance vol IV, part B+C
Csoil = DOSEpres/Depth*RHO*k-DOSEpres/ Depth*RHO*k*exp(-T*k)	mg/Kgwwtsoil	PECsoildrift	6,75E-03	O	According to TMIII2013
Predicted concentration in porewater	µg/L	PECgroundwater	2,68	O	

With 99% drift elimination					
Dose of a.i. deposited to soil	g.m-2.h-1	DOSEpres	4,23E-09	O	with degradation
Dose of a.i. deposited to soil	mg.m-2.d-1	DOSEpres	1,02E-07	O	with degradation
Depth of the receiving soil compartment	m	Depth	0,2	D	Default value based on BPR guidance vol IV, part B+C
Density of wet bulk soils	(kg/m3)	RHO	1700	D	Default value based on BPR guidance vol IV, part B+C
first order rate constant for removal (12 ºC)	(d-1)	k	2,87E-03	D	Default value based on BPR guidance vol IV, part B+C
Csoil = DOSEpres/Depth*RHO*k-DOSEpres/ Depth*RHO*k*exp(-T*k)	mg/Kgwwtsoil	PECsoildrift	6,75E-05	O	According to TMIII2013
RELEASEair	kg.d-1	RELEASEair	3,05E-04		
Pedicted concentration in porewater	µg/L	PECgroundwater	0,027	O	

PEC_{soil} (indirect exposure to soil from sewage sludge application to land)

As mentioned above, direct release to soil is not of concern. The only exposure route for soil regarding Bronopol is the application via sewage sludge. The concentration of active substance in dry sewage sludge can be calculated using equations taken from the ECHA guidance on ERA (2017, p.73ff) plus default parameters presented in the same guidance document, which are shown below.

Table 127: Default values and input parameters for calculating the bronopol concentrations reaching the STP via the respective PT.

PARAMETER			VALUE
SUSPCONC$_{inf}$	concentration of suspended matter in STP influent	Default (Table 7, ERA 2017)	0.45 kg m^{-3}
EFFLUENT$_{stp}$	effluent discharge rate of STP	default (Based on default values of Table 7, ERA 2017)	2.0E+06 L.d^{-1} (2,000 m^3 d^{-1})
SURPLUSsludge	surplus sludge per inhabitant equivalent	default TAB ENV 239	0.0212 kg.d^{-1} eq^{-1}
CAPACITY$_{stp}$	capacity of STP	default (Table 7, ERA 2017)	10,000
WASTEW$_{inhab}$	Sewage flow per inhabitant	default (Table 7, ERA 2017)	200 L.d^{-1} eq^{-1}
SLUDGERATE	rate of sewage sludge production	calculated	813 kg.d^{-1}
APPL.RATE TO ARABLE LAND		default (Table 9, ERA 2017 and TAB ENV 36)	5000 kg.ha^{-1}.yr^{-1}
APPL.RATE TO GRASSLAND		default (Table 9, ERA 2017 and TAB ENV 36)	1000 kg.ha^{-1}.yr^{-1}
Fstp$_{sludge}$	fraction of emission directed to sludge by STP (SimpleTreat v4.0)	output	0.0122
log K_{ow}	octanol-water partition coefficient	Input (Data set)	-0.42
DT50$_{soil}$	Half-life in soil	Output (Table 6, ERA 2017)	300 days
K_{OC}	Organic carbon partition coefficient	Input (Data set)	136 L.kg^{-1}

Based upon the relevant physico-chemical properties of Bronopol, SimpleTreat v4.0modelling predicts limited adsorption (1.22 %) onto sewage sludge. 2.8 % of Bronopol which reaches the STP are expected to be discharged to surface water. 95.97 % degradation occurs within the STP. The predicted $E_{localSTP}$ is calculated according to the respective ESD. A calculation of C_{sludge} was carried out for all PTs based on $E_{localSTP}$ and ERA EQ. 39 (2017). Finally, the corresponding concentrations of Bronopol reaching the farmland ($C_{localsoil}$) via sludge application for arable land, grassland as well as for the ecosystem were calculated according to ERA EQ. 66 (2017) assuming 180 days for arable- and for grassland and 30 days for the ecosystem (Table 9, ERA 2017).

Consequently, small indirect releases of bronopol to agricultural land can be predicted by application of STP sludge. The assumption is generally made that such sludge is applied annually for 10 years.

The degradation studies carried out on activated sludge have demonstrated that bronopol cannot be regarded as readily biodegradable but it is a borderline case. Considering this and the very low log K_{ow} of -0.42, a low Kp of 2.72, and a very high degradation rate in natural waters (supporting information from published literature), and according to the supporting information regarding readily biodegradability (A7.1.1.2.1_01), which indicates that bronopol was biotransformed by day 29 to 92 % (ECHA Guidance R7b: "when results of ready biodegradablity tests indicate that the pass level criterion is almost fulfilled, such results can be used as evidence for inherent biodegradability"), a half-life of 300 days in soil can therefore be applied (Table 6, ERA 2017). The DT50 of 300 days was also used to assess the risk to ground water. At product authorization stage DT50 value of 300 days shall be used as a worst-case value unless new, reliable data are provided allowing derivation of DT50 value for soil. This would crudely represent the removal rate of Bronopol from top soil via degradation.

The Csludge values are reported below:

	Csludge
PT2	1,10E+00
PT11	
small open cont	2,11E-01
small open shock	9,15E-01
close drainage	2,25E+00
close degrad	
PT12	
small paper	9,38E+00
typical case	2,76E+01
Worst case STP	1,35E+02

PEC in groundwater

A mean K_{OC} value of 136 $L.kg^{-1}$ suggests that Bronopol has the potential to be mobile in soil and therefore it may lead to indirect exposure of groundwater.

Based upon results presented above, and ECHA guidance for risk assessment, which indicates that it is more appropriate to base groundwater assessment upon TWA concentrations in soil over 180 d using endpoints derived for arable land and grassland, PEC gw are estimated. The tier 1 calculation for the PEC gw in several uses represents a concentration in porewater of non-specific "agricultural soil" significantly above the current quality standard set at 0.1 $\mu g.L^{-1}$ by the EU Drinking Water Directive (98/83/EC) and indicates the need for additional FOCUS groundwater modelling.

A small summary of the PEC gw obtained without refinement is included below:

Table 128: Summary table on calculated PEC gw values for Bronopol

	Elocal	PEC gw (threshold value 0.1 µg/L)
PT2	7.33E-02	0.77
PT11		
small open cont	1.41E-02	0.15
small open shock	6.09E-02	0.64
close degrad	1.29E-05	1.36E-04
PT12 small	2.32E-01	6.59
PT12 typical case	3.87E+00	19.38
PT12 worst case STP	1.90E+01	94.92

Only the following uses do not need a refinement with FOCUS:

- PT11 closed system with degradation.

FOCUS PEARL requires an application rate in $kg.ha^{-1}$ as input parameter.

According to TAB entry ENV 36:

"In case of running sewage sludge application scenarios in FOCUS groundwater models it was agreed at WG-II-2014 that both grassland (alfalfa) and agricultural land (maize) should be used. In case of grassland application, the scenario considers one sewage sludge application per year on 1st of March (absolute application) and 10 cm incorporation depth. In case of agricultural land application, the scenario considers one sewage sludge application per year to maize 20 days before crop event "emergence" (relative application) and 20 cm incorporation depth. The application rate of the active substance Appl_rateagr/grass [kg/ha] at one application date as input parameter in FOCUS groundwater models is calculated by:

$$Appl\_rate_{agr/grass} = App_{sewage\_sludge\_agr/grass} \times C_{sludge} \times 10^{-6}$$

Where:
Appsewage_sludge_agr = annual sewage sludge application rate on agricultural land = 5,000 kg/ha
Appsewage_sludge_grass = annual sewage sludge application rate on grassland = 1,000 kg/ha
Csludge = concentration of a.s. in dry sewage sludge [mg/kg] (ref. to eq. 39 in guidance BPR IV B v.2.0).

$$C_{sludge} = \frac{Fstp_{sludge} \cdot Elocal_{water} \cdot 10^6}{SLUDGERATE}$$ Equation 39

Explanation of symbols

$Elocal_{water}$	local emission rate to water during episode	[kg · d^{-1}]	**Equation 5**
$Fstp_{sludge}$	fraction of emission directed to sludge by STP	[-]	Estimation by EUSES/Simple treat
SLUDGERATE	rate of sewage sludge production	[kg · d^{-1}]	**Equation 38**
C_{sludge}	concentration in dry sewage sludge	[mg · kg^{-1}]	

Hence, the output of this equation 39 and the application rates are summarized below:

Table 129: Summary table on calculated Csludge and application rates

	Csludge	Appl rate arable	Appl rate grass
PT2	1,10E+00	5,51E-03	1,10E-03
PT2 metabolite	1,10E+00	5,51E-03	1,10E-03
PT11			
small open cont	2,11E-01	1,06E-03	2,11E-04
small open shock	9,15E-01	4,58E-03	9,15E-04
close drainage	2,25E+00	1,13E-02	2,25E-03
close degrad			
PT12 small	9,38E+00	4,69E-02	9,38E-03
PT12 typical case	2,76E+01	1,38E-01	2,76E-02
PT12 worst case STP	1,35E+02	6,76E-01	1,35E-01

Table 130: Required input parameters for FOCUS pearl 4.4.4.

Model used	FOCUS PEARL 4.4.4
Years of simulation	26 (including 6 yrs "warming up" period)
Application rate for combined emissions (kg.ha^{-1})	TAB ENV 36
Standard crop	Arable land: Maize Grassland: Alfalfa
Application depth	Incorporation 20 cm (Maize) 10 cm (Alfalfa)
Date of application	Maize: One application per year, 20 days before crop emergence Alfalfa: One application per year on the 1st of March
Molar mass	199.99 g.mol^{-1}
Vapour pressure	5.1E-03 Pa at 12 °C
Water solubility	3.04E+05 mg.L^{-1} at 20 °C
Kom	136 / 1.724 L.kg^{-1} = 78.9 at 20 °C
Freundlich exponent, 1/n	1*
DT_{50}soil	300 d at 12 °C
Coefficient for uptake for plant	0
Molar activation energy	65.4 kJ.mol^{-1}

*According to TAB ENV 22, and as the Freundlich isotherms including 1/n was based on only four concentrations, although bronopol seems to be moderately mobile, a default 1/n of 1 is to be used in any FOCUS modelling (case 2). This more conservative value is used to cover the uncertainty in the relationship between the substance's sorption and concentration.

As from the tier 1 calculation there remains risk for groundwater from several scenarios, the scenarios that leads to the highest calculated groundwater concentrations is calculated. This decision was made on the input parameter Elocal.

In the following tables, the results from FOCUS Pearl 4.4.4 for application rates derived from several uses are provided:

Table 131: PT11 small open continuous 80th percentile annual average concentration in groundwater, using FOCUS model PEARL 4.4.4. [µg.L^{-1}].

Scenario	Grassland	Arable land
Châteaudun	0.010834	0.058285
Hamburg	0.015306	0.082386
Jokioinen	0.014713	0.060776
Kremsmünster	0.010376	0.065091
Okehampton	0.011712	0.060459
Piacenza	0.010726	0.031398
Porto	0.006519	0.017196
Sevilla	0.006934	0.061609
Thiva	0.008234	0.058285

Table 132: PT11 small open shock 80th percentile annual average concentration in groundwater, using FOCUS model PEARL 4.4.4. [µg.L^{-1}].

Scenario	Grassland	Arable land
Châteaudun	0.046981	0.251837
Hamburg	0.066375	**0.355969**
Jokioinen	0.063801	
Kremsmünster	0.044996	0.262600
Okehampton	0.050790	0.281242
Piacenza	0.046513	0.261227
Porto	0.028270	0.135661
Sevilla	0.030071	0.074299
Thiva	0.035705	0.266197

Table 133: PT12 small paper factories 80th percentile annual average concentration in groundwater, using FOCUS model PEARL 4.4.4. [µg.L^{-1}].

Scenario	Grassland	Arable land
Châteaudun	0.481622	2.578853
Hamburg	0.680434	**3.645188**
Jokioinen	0.654048	2.689067
Kremsmünster	0.461269	2.879965
Okehampton	0.520671	2.675013
Piacenza	0.476821	1.389195
Porto	0.289805	0.760839
Sevilla	0.308270	2.725901
Thiva	0.366023	2.578853

Table 134: PT2 80th percentile annual average concentration in groundwater, using FOCUS model PEARL 4.4.4. [µg.L^{-1}].

Scenario	Grassland	Arable land
Châteaudun	0.0564800	0.302974
Hamburg	0.0797950	**0.428251**
Jokioinen	0.0767010	0.315922

Scenario	Grassland	Arable land
Kremsmünster	0.0540930	0.33835
Okehampton	0.0610590	0.314271
Piacenza	0.0559170	0.163208
Porto	0.0339860	0.089386
Sevilla	0.0361510	0.32025
Thiva	0.0429240	0.302974

The FOCUS results give values above the threshold value of current quality standard of 0.1 µg/L, EU Drinking Water Directive (98/83/EC) for PT2, PT11 (small shock and close drainage) and PT12 (small paper factories). For such cases, the values for PEC_gw_arable land are not far above the trigger value. Therefore, a qualitative risk assessment is considered. Bronopol is known to degrade both abiotic and biotically, the realistic amount of a.s. reaching the STP is likely to be much lower than estimated in the scenarios. Further, there is data available from several monitoring studies, showing that no Bronopol has been detected in environmental compartments, including sludge.

The LOD in the Swedish monitoring study for sludge was 12 -24 µg/kg dwt (see section A.4.1.4 monitoring data). Bronopol could not be detected in sludge and therefore its concentration was in all cases < LOD. A LOD of 12-24 µg/kg dwt is of similar order of magnitude than the lowest Clsudge values we got which are between 0.01 to 1 mg/kg. With a Csludge below 0.1 mg/kg there is no risk to groundwater. Still, taking the upper LOD with 24 µg/kg dwt as input parameter for Csludge, the resulting application concentrations are: Arable: 1.20E-4 kg/ha and Grass: 2.40 E-5 kg/ha. This leads to groundwater concentrations according to FOCUS Pearl 4.4.4 of 0.006 µg/L for arable and 0.001 µg/L for grass. As a result, it can be concluded that bronopol does not pose any risk for the groundwater compartment. Thus, the risks are considered as acceptable.

Literature monitoring research data

In 2005, the Swedish environmental research institute conducted an assignment to investigate the environmental concentrations of chemicals. The aim of the sub report was to determine concentrations of biocides in several compartments. In the Table below **¡Error! No se encuentra el origen de la referencia.** the sampling strategy is shown. Municipal sewage sludge was also investigated for residues. De-watered sludge was stored in a freezer (18°C) until GC-MS analysis. For detailed methods please refer to the report.

Here is presented the sampling strategy, including sampling sites and (environmental)compartments

that were sampled:

		Air	Precipi-tation	Water	Sediment	Biota	Sludge	Provisions	Urine
Back-ground	Pallas	x							
	Råö	x	x						
	Lilla Öresjön			x	X	x			
	Stensjön			x	X	x			
	Tärnan			x	X	x			
Diffuse	Stockholm	x	x						
	Riddarfjärden			x	X	x			
	Stora Essingen			x	X	x			
	Årstaviken			x	X	x			
	Mälaren				X				
	Municipal STPs			x			x		
	Landfills			x					
Point source	Industrial plants Harbor	x		x	X				
Human exposure								x	x

The LODs for bronopol at the respective compartment is provided below:

Matrix	Limit of Detection
Air	0.05-0.07 ng.m^{-3}
Precipitation	0.16 µg.L^{-1}
Water	0.05 µg.L^{-1}
Sediment	10-17 µg.kg^{-1} dwt
Fish	10 µg.kg^{-1} fw
Sludge	12-24 µg.kg^{-1} dwt
Food stuffs	10 µg.kg^{-1} fwt

In all samples, the concentrations of bronopol were below the LOD. Leading to the conclusion that bronopol could not be detected in any of the above-mentioned compartments. Therefore, exposition of soil via STP sludge application under realistic conditions is unlikely. This supports the qualitative assessment that considers that Bronopol degrades both abiotic and biotically and much less Bronopol than expected will reach the STP. No Bronopol has been detected in the sludge, hence the risks to groundwater compartment can be considered much lower than the values estimated.

Please see section A.4.1.4 and C.2.5 for further information.

B.4.4 Primary and Secondary poisoning

Waiving argument

Primary and Secondary poisoning

Bronopol does not accumulate in biota. This is apparent from its low log P_{ow} of 0.17 (experimental value at pH 4 (not stable at pH 7), calculated values -0.42). Based on the low partition coefficient it was not required to determine any bioconcentration factors (BCF) for Bronopol. Estimations by QSAR yielded BCFs ≤ 1 for aquatic and terrestrial ecosystems, confirming no potential for bioaccumulation.

B.5 Assessment of effects on Human Health for the product

B.5.1 Product(s)

The biocidal products [redacted], [redacted] and [redacted] all consist of ≥98% w/w Bronopol. Since this Assessment report contains information merged from two separate dossiers originally submitted by three applicants, these three biocidal products are covered in one representative product in the following. Further information on the individual/representative product(s) are given in section B.1

As the biocidal products and representative product are identical with the active substance, reference is made to data provided in the active substance part of this dossier. It is considered that toxicity and resulting classification are identical for the representative product.

B.5.2 Dermal absorption

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

Value(s) used in the Risk Assessment – Dermal absorption	
Value	100% bronopol (powder) - 10% (default value for solids) >5-30% bronopol - 10% (default value for concentrated water-based formulations) ≤5% bronopol - 50% (default value for diluted water-based formulation)
Justification for the selected value(s)	Based on EFSA Guidance for dermal absorption.

B.5.3 Acute toxicity

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

B.5.3.1 Overall conclusion on acute toxicity

Value used in the Risk Assessment – Acute toxicity	
Value(s)	Oral route: LD50 = 211 mg/kg bw (males), 193 mg/kg bw (females) Dermal route: LD50 = 1600 mg/kg Inhalation route: 0.588 mg/L/4h < LC50 < 1.14 mg/L/4h
Justification for the selected value(s)	Based on toxicological information of the active substance, identical to the representative product.
Classification for the product according to CLP	Oral route: Bronopol is classified as Acute tox Cat.3 H301 under Regulation (EC) No 1272/2008. Dermal route: Bronopol is harmonised classified as Acute tox Cat. 4 H312 under Regulation (EC) No 1272/2008 (ATP 1 to CLP). Inhalation route: Bronopol is classified as Acute tox Cat.3 H331 under Regulation (EC) No 1272/2008.

B.5.4 Corrosion and irritation

B.5.4.1 Skin corrosion and irritation

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

Conclusion used in the Risk Assessment – Skin corrosion/irritation	
Conclusion	The observed clinical signs from the available skin corrosion and irritation studies are indicative of skin irritation and are addressed by the classification as Skin Irrit. 2 H315.
Justification for the conclusion	Based on toxicological information of the active substance, identical to the representative product.

B.5.4.2 Serious eye damage and eye irritation

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

Conclusion used in the Risk Assessment – Serious eye damage/Eye irritation	
Conclusion	The observed clinical signs from the available eye damage and eye irritation studies are indicative of serious eye damage and are addressed by the classification as Eye Dam. 1 H318.
Justification for the conclusion	Based on toxicological information of the active substance, identical to the representative product.

B.5.4.3 Respiratory tract irritation

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

Conclusion used in the Risk Assessment – Respiratory tract irritation	
Conclusion	The observed clinical signs from the available acute inhalation toxicity studies are indicative of respiratory irritation and are addressed by the classification as STOT SE 3 H335.
Justification for the conclusion	Based on toxicological information of the active substance, identical to the representative product.

B.5.4.4 Overall conclusion on corrosion and irritation

Conclusion used in the Risk Assessment – Corrosion and irritation	
Value(s) or Conclusion(s)	Bronopol causes skin and respiratory tract irritation and eye damage.
Justification for the selected value/ conclusion	Based on toxicological information of the active substance, identical to the representative product.
Classification of the product according to CLP	Skin irritant Cat. 2, H315 Eye damage Cat. 1, H318 STOT SE Cat. 3, H335

B.5.5 Sensitisation

B.5.5.1 Skin sensitisation

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

Conclusion used in the Risk Assessment – Skin sensitisation	
Value/ Conclusion	Not skin sensitising.
Justification for the selected value/ conclusion	Based on toxicological information of the active substance, identical to the representative product.
Classification of the product according to CLP	No classification proposed.

B.5.5.2 Respiratory sensitisation

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

B.5.5.3 Overall conclusion on sensitisation

Conclusion used in the Risk Assessment – Sensitisation	
Conclusion(s)	No data on respiratory sensitisation is available. As there are currently no appropriate tests available and there is no indication of respiratory sensitisation effects for Bronopol, respiratory sensitisation does not need to be taken into account.

Justification for the conclusion(s)	Based on toxicological information of the active substance, identical to the representative product.
Classification of the product according to CLP	No classification proposed.

B.5.5.4 Other

The representative product is identical with the active substance, hence no further data are included in this part. Please refer to the studies included in Part A.

B.6 Environmental effects assessment for the product

The ecotoxicological properties of the product are identical to the properties of the active substance. The product does not contain any additional compound that adversively affect the conclusions of the risk assessment for the active substance. Therefore, no further assessment for the product is needed.

Information on the ecotoxicity of the active substance is presented in Part A, Section A.4.2.1 to A.4.2.7.

The environmental risk assessment is provided in Part B (exposure assessment in section B.4) and Part C (risk characterisation in section C.2).

B.6.1 Atmosphere

Not relevant.

B.6.2 STP

Not relevant.

B.6.3 Aquatic compartment

Not relevant.

B.6.4 Terrestrial compartment

Not relevant.

B.6.5 Primary and Secondary poisoning

Not relevant.

C. Risk characterisation of the biocidal product(s)

C.1 Risk Characterisation for human health

C.1.1 Critical endpoints

C.1.1.1 Systemic effects

Acute effects				
Study	**Route and doses**	**Relevant effects**	**NOAEL/ LOAEL**	**References**
Acute oral toxicity study, rat	Oral gavage 100, 150 (females only), 200, 300 (males only) mg/kg bw	Signs of gastrointestinal irritation, Mortality (most deaths within 24 h)	LD50 = 211 mg/kg bw (males) LD50 = 193 mg/kg bw (females)	A6_01_1-1
Acute dermal toxicity, rat	Dermal Groups of ten male rats. Dose: 25, 100, 400 and 1600 mg/kg, respectively.	Animals at all dosages: damage to the treated area of skin dark perianal stains. 3 rats given 1600 mg/kg died.	LD50 = 1600 mg/kg bw	A6.01.2_02
Acute inhalation toxicity, rat	a) Inhalation (nose only) 120, 1140 mg/m³ b) Nominal concentrations: 0, 1.80, 2.59, 23.23 mg/L Analytical concentrations: 0, 0.038, 0.089, 0.588 mg/L.	Mortality	a) 120 mg/m³ < LC50 <1140 mg/m³ b) LC50 for males and females > 0.588 mg/L (equivalent to > 588 mg/m³) Conclusion: 0.588 mg/L/4h < LC50 < 1.14 mg/L/4h	a) A6_01_3-1 b) A6.01.3_01
Sub-chronic oral toxicity study, dogs	Oral gavage 0, 4, 8, 20 mg/kg bw/day	Cinical signs, vomiting 30 min after each dose	NOAEL = 8 mg/kg bw	A6.04.1_02

Medium-term effects				
Study	**Route and doses**	**Relevant effects**	**NOAEL/ LOAEL**	**References**
Sub-chronic oral toxicity study, rats	Oral, drinking water 0, 60, 250, 1000 ppm, equivalent to 0, 6.2, 24.3, 83.9 mg/kg bw/day (males) and 0, 6.8, 25.5, 86.0 mg/kg bw/day (females)	Changes in haematology and clinical pathology parameters (reduced Hb and MCVC (males), reduced urine volume with increased osmolarity), reduced water consumption, changes in kidney (increased weight, histopathology)	NOAEL = 24.3/25.5 mg/kg bw/day for males/females	A6_04_1-1
Sub-chronic oral toxicity study, rats	Oral, drinking water 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)	Reduced bw development, food and water consumption; changes in kidney (increased weight, nephropathy)	NOAEL = 59.2/136.3 mg/kg bw/day for males/females	A6_04_1-2
Sub-chronic oral toxicity study, rats	Oral gavage 20, 80, 160 mg/kg bw/day	Mortality and reduced bw gain (mid and high dose), Respiratory distress and abdominal distention, histopathological changes in kidney	NOAEL < 20 mg/kg bw/day	A6.04.1_01
Sub-chronic oral toxicity study, dogs	Oral gavage 4, 8, 20 mg/kg bw/day	Increased absolute liver and spleen weights, however, no related gross pathological and histopathological changes	NOAEL = 8 mg/kg bw/day	A6.04.1_02

Long-term effects seen in studies				
Study	**Route and doses**	**Relevant effects**	**NOAEL/ LOAEL**	**References**
Chronic oral toxicity study, rat	Oral, drinking water 10, 40, 160 mg/kg bw/day	Reduction in food consumption and body weight gain, increased mortality, reduction in grooming activity	NOAEL = 10 mg/kg bw/day	A6.07_01_a
Chronic dermal toxicity study, rat	Dermal 0.2, 0.5% corresponding to 20, 50 mg/kg bw/day (assuming a body weight of 30 g/mouse)	Reduced body weight gain	NOAEL = 0.2% (20 mg/kg bw/day)	A6.07_02_a
2-generation toxicity study, rat	Oral via drinking water 0.01, 0.05, 0.15% equivalent to target doses of 0, 10, 50, 150 mg/kg/day of test material	Reduced bw gain (gestation), water consumption, increased rel. kidney and thyroid weight, minor microscopic changes in kidneys, thyroid, stomach and liver	NOAEL = 10 mg/kg bw/day	A6_08_2-1

C.1.1.1 Local effects

Route	**Study (reference) Test substance**	**Relevant effects NOAEC/LOAEC**	**Classification**	**Hazard category[1]**
Dermal	a) Acute dermal irritation (A6.01.4_01) b) Acute dermal toxicity study (A6.01.2_01) Bronopol	a) Irritating to skin of rabbit b) Cutaneous effects at the application site (white discoloration of the skin, erythema, edema, eczema-like skin change, scaling and crust formation) at 2000 mg/kg bw	Skin irritant Cat. 2 (H315)	Low
Respiratory	a) Acute inhalation toxicity study (A6_01_3-1) b) Acute inhalation toxicity study (A6.1.3_01) Bronopol	a) Bloody nose and mouth breathing (at 1140 mg/m^3 only) as well as labored breathing (at 120 and 1140 mg/m^3) b) Nasal discharge, red staining and inflammation of the eyes and staining of the head, accompanied by swelling of the head, throat and/or the forepaws at 0.588 mg/L	STOT SE Cat. 3 (H335)	Low

Route	Study (reference) Test substance	Relevant effects NOAEC/LOAEC	Classification	Hazard category[1]
Oral	Acute oral toxicity (A6_01_1-1) Bronopol	Signs of gastrointestinal irritation	-	-

[1]According to ECHA guidance Vol III Part B.

C.1.1.2 Absorption

Route	Study	Test substance and concentration of a.s.	Value
Oral	A6.02_01, A6.02_02, A6.02_03, A6.02_05	Based on the results of various studies, rapid and complete absorption (≥80% of applied dose) of Bronopol after oral exposure is concluded	100%
Dermal	A6_02-4	2-^{14}C Bronopol (specific activity: 15.4 µCi/mg, radiochemical purity: 99%) was dermally applied to rats at 10 mg/kg bw corresponding to 0.42-0.45 mg/cm^2 For water-based mixtures containing bronopol, for which there is no information, the default values should be used.	43.6% 100% bronopol (powder): 10% (default value for solids); >5-30% bronopol: 10% (default value for concentrated water-based formulations); ≤5% bronopol: 50% (default value for diluted water-based formulation)
Inhalation	Default (in the absence of (substance-)specific information)	n.a.	100%

C.1.2 Reference values

C.1.2.1 Reference values to be used in Risk Characterisation

The following reference values do not cover possible genotoxicity.

Studies selected for reference value derivation		
Reference value	**Study**	**Rationale for selecting the study**
AEL	90-day oral toxicity study in dogs (A6.4.1_02)	The sub-chronic toxicity study in dogs (A6.4.1_02), although was only considered similar to guideline, shows relevant effects. Therefore, the NOAEL of 8 mg/kg bw/day based on systemic toxicity as increase in liver and spleen weights and vomiting in this 90-day oral toxicity study in dogs is considered the most conservative key value for the AEL. Since the systemic effects observed in the short- and long-term studies are secondary to the reduction in water intake and the severity of those observed in the medium-term studies does not change with exposure time, a single AEL has been derived.
ARfD*	90-day oral toxicity study in dogs (A6.04.1_02)	A NOAEL of 8 mg/kg bw/day based on vomiting observed 30 min after each dose observed in this 90-day repeated dose toxicity study in dogs is considered the most conservative key value for the acute reference dose level.
ADI**	90-day oral toxicity study in dogs (A6.4.1_02)	The sub-chronic toxicity study in dogs (A6.4.1_02), although was only considered similar to guideline, shows relevant effects. Therefore, the NOAEL of 8 mg/kg bw/day based on systemic toxicity as increase in liver and spleen weights and vomiting in this 90-d oral toxicity study in dogs is considered the most conservative key value for the ADI.
AEC_{inhal}short-term	Acute inhalation toxicity study (A6.1.3_01)	The local effects observed in the acute inhalation toxicity study are considered the most relevant local, acute type effect. Moreover, this study can be used to derive a key value for the AEC.

Studies selected for reference value derivation		
Reference value	**Study**	**Rationale for selecting the study**
$NOAEC_{irrit}$	Patch test in humans (A6_01_5-4; A6.12.6_03)	For the characterization of local effects, a NOAEC of 0.5% has been considered from a sensitization study in humans under the basis of irritative reactions with an irritation rate of 18% at the higher dose (1% mg/L).

*According to the ECHA Guidance Vol III Part B+C v.4 (2017), "For certain PTs and use patterns, especially if the active substance can enter the food chain, ADI and, if necessary, ARfD should be derived". Due to the relatively marked acute toxicity and as acute exposure scenarios may not be excluded for humans, an acute reference dose (ARfD) may be required for the risk assessment of Bronopol. While the ARfD should describe a single exposure scenario, no study with single exposure is available where also all potentially relevant endpoints were examined. Therefore, also sub-acute/-chronic toxicity studies are used for the assignment of an ARfD. As these are essentially the same studies as for the AEL, the ARfD also results in 0.08 mg/kg bw for Bronopol.

While primary exposure to Bronopol may be limited to sub-chronic exposure (workers in the production of biocidal products), chronic secondary exposure may not be excluded due to the use pattern of the biocidal products. Therefore, an ADI is presented to provide a theoretical basis for chronic risk assessment considerations.

**An ADI = 0.02 mg/kg bw/d has been set under Reg. 1107/2009. However, this value has not been assessed by any eCA since it was extracted from a summary of an EMA CVMP report in 2001. It was calculated by applying a safety factor of 200 to the NOAEL of 20 mg/kg bw/d, which was established in the 13-week repeated dose toxicity study in dogs. A safety factor of 200 was chosen to compensate for the limited range of clinical chemistry investigations carried out in this study and the lack of analysis of the dosing solution. Under BPR, a safety factor of 200 does not seem justified in this study.

Reference value	**NOAEL (LOAEL) NOAEC/LOAEC**	**AF**	**Correction for oral absorption**	**Value**
AEL	8 mg/kg bw/day ($NOAEL_{oral}$)	100	n.a.	0.08 mg/kg bw/day
ARfD	8 mg/kg bw ($NOAEL_{oral}$)	100	n.a.	0.08 mg/kg bw/day
ADI	8 mg/kg bw/day ($NOAEL_{oral}$)	100	n.a.	0.08 mg/kg bw/day
AEC_{inhal}short-term	89 mg/m³ ($NOAEC_{inhal}$)	25	n.a.	11 mg/m³
$NOAEC_{irrit}$	0.5% ($NOAEC_{irrit}$)	-	n.a.	0.5%

C.1.2.2 Uncertainties and assessment factors

Systemic reference values

AEL		
Uncertainty	**AF**	**Justification**
Interspecies variability	10 (4 x 2.5)	Default
Intraspecies variability	10 (3.16 x 3.16)	Default
Route to route extrapolation	1	-
Time duration extrapolation	1	-
NOAEL to LOAEL extrapolation	1	-
Dose response	1	-
Severity of key health effects	1	-
Overall AF	**100**	(n.a.)

ARfD		
Uncertainty	**AF**	**Justification**
Interspecies variability	10 (4 x 2.5)	Default
Intraspecies variability	10 (3.16 x 3.16)	Default
Route to route extrapolation	1	-
Time duration extrapolation	1	-
NOAEL to LOAEL extrapolation	1	-
Dose response	1	-
Severity of key health effects	1	-
Overall AF	**100**	(n.a.)

ADI		
Uncertainty	**AF**	**Justification**
Interspecies variability	10 (4 x 2.5)	Default
Intraspecies variability	10 (3.16 x 3.16)	Default
Route to route extrapolation	1	-
Time duration extrapolation	1	-
NOAEL to LOAEL extrapolation	1	-
Dose response	1	-
Severity of key health effects	1	-
Overall AF	**100**	(n.a.)

Local reference values

AEC_{inhal} short-term		
Uncertainty	**AF**	**Justification**
Interspecies variability	2.5	For AEC determination, as far as only local effects were observed, a refined interspecies factor is proposed in line with the recommendations given in the ECHA Guidance on information requirements and chemical safety assessment (Chapter R.8). It can be assumed that for a local effect at the port of entry, toxicokinetics do not contribute significantly to interspecies differences. In contrast, as the mechanism is not clearly known, it is prudent to assume that the toxicodynamic component should be kept at 2.5.
Intraspecies variability	10	It can be assumed that for a local effect at the port of entry, toxicokinetics do not contribute significantly to intraspecies differences. In contrast, as the mechanism is not clearly known, it is prudent to assume that the toxicodynamic component should be kept at 10.
Route to route extrapolation	1	-
Time duration extrapolation	1	-
NOAEC to LOAEC extrapolation	1	-
Dose response	1	-
Severity of key health effects	1	-
Overall AF	**25**	(n.a.)

C.1.2.3 Maximum residue limits or equivalent

Default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg. 396/2005.

C.1.2.4 Specific reference value for groundwater

Reference value for Bronopol in groundwater: 0.1 µg/L.

C.1.3 PT02

C.1.3.2. Professional uses

Systemic effects

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 2 Exposure during application of biocidal product to the waste holding tank and emptying of collecting tanks after usage	1/none (Bronopol)	0.08	1.69E-01	211	**No**
	1/none (Formaldehyde)	0.15	8.89E-03	5.93	Yes
	2/ PPE (Bronopol)	0.08	2.01E-03	2.56	Yes
	2/ PPE (Formaldehyde)	0.15	1.47E-04	0.098	Yes
Scenario 3 Exposure during emptying of collecting tanks after usage	1/none	0.08	4.00E-04	0.5	Yes
	2/ PPE	0.08	4.00E-05	0.05	Yes

Combined scenarios

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario [2, 3]	1/none	0.08	1.69E-01	242	**No**
	2/ PPE	0.08	2.05E-03	2.93	Yes

When the end-product is applied the estimated systemic exposure of professional worker is also considered acceptable when PPEs are used (protective gloves).

Finally, when the tank is emptied the estimated systemic exposure of professional worker is considered acceptable without PPEs.

Assuming one person is performing all tasks, the combined exposure was estimated. The estimated systemic exposure of professional worker is below the reference value only when protective gloves are worn.

Due to irritant properties of the end-product any exposure to the end-product must be prevented using the technical and organizational RMM adequate for high and low hazard chemicals and appropriate PPE must be used (see next chapter for details).

Local effects

According to the criteria of the Regulation 1272/2008, Bronopol dilutions are proposed to be classified as:

1. 30% dilution: a severe eye irritant category 1 (H318), an irritant to skin category 2 (H315) and an irritant to respiratory tract category 3 (H335)
2. 10% dilution: a severe eye irritant category 1 (H318) and an irritant to skin category 2 (H315)
3. <10% dilution: not classified.

Therefore an as AECs can be set, quantitative and qualitative assessment of local effects will be performed in Section B.

Quantitative risk assessment

Task/ Scenario	Tier	AEC_{inhal}short-term mg/m^3	Estimated uptake mg/m^3	Acceptable (yes/no)
Scenario 2. **Application**	1	3.6	1.96E-03	Yes
Scenario 3. **Application**	1	3.6	1.02E-08	Yes

This results in a mean event concentrations and peak concentrations which are below the AEC_{inhal}short-term of 3.6 mg/m^3. Taking this into account, the bronopol evaporation vapours exposure to is considered acceptable.

Task/ Scenario	Tier	$NOAEC_{irrit}$ % (ppm)	Estimated uptake % (ppm)	Acceptable (yes/no)
Scenario 2. **Application**	1	0.5 (5000)	10 (100000)	**No**
Scenario 3. **Application**	1	0.5 (5000)	0.01 (100)	Yes

This results in a mean event concentrations and peak concentrations which are above the $NOAEC_{irrit}$ of 0.5% with the exception of scenario 3. Taking this into account, the bronopol dermal exposure to is considered acceptable when workers wears PPE (protective gloves and coveralls).

Qualitative risk assessment

Primary Exposure / Professional use – Use of the concentrated product and diluted products

Hazard			Exposure							Risk
Hazard Category	**Effects in terms of C&L**	**Additional relevant hazard information**	**PT**	**Who is exposed?**	**Tasks, uses, processes**	**Potential exposure route**	**Frequency and duration of potential exposure**	**Potential degree of exposure**	**Relevant RMM&PPE**	**Conclusion on risk**
High	Eye Dam, Cat. 1 (H318)	Concentration ≥10%	2	Professional users	Preparation of final end-use product formulations from biocidal product Application of final end-use product biocidal product to the waste holding tank and emptying of collecting tanks after usage	Eyes	Few minutes per day or less	≥ 10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Containment as appropriate; - Segregation of the emitting process; - Effective contaminant extraction; - Good standard of general ventilation; - Minimisation of manual phases; - Regular cleaning of equipment and work area; - Avoidance of contact with contaminated tools and objects; **Organisation** - Minimise number of staff exposed; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** • Chemical goggles	Acceptable: + Minimisation of manual phases. + Low frequency + Used for short duration + Low amount used per event; + Professionals using PPE; + Professionals following instructions for use; Good standard of personal hygiene.

Low	Skin irrit, Cat. 2 (H315)	≥ 10%				Skin	More than few minutes but equal to or less than few hours per day.	≥10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Minimisation of manual phases/work tasks; - Minimisation of splashes and spills; - Avoidance of contact with contaminated tools and objects; - Regular cleaning of equipment and work area; **Organisation** - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** - Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure)	
	STOT SE, Cat. 3 (H335)	≥ 30%				RT		≥ 30%		

The end-products (30% a.s. and 10% a.s.) has been allocated to:

1. the "High" hazard category according to the classification as a severe eye irritant (Eye Dam 1 - H318) and
2. the "Low" hazard category according to the classification as an irritant to skin (Skin Irrit 2 – H315) and/or an irritant to respiratory tract (STOT SE 3 – H335)

In the hazard categories proposed in the Guidance for Human Health Risk Assessment & evaluation (Volume III – Part B + C). The biocidal product is diluted to the recommended treatment concentration (30% a.s. or 10% a.s.) before use. The application task involves (1) replacing chemical toilet product (30% or 10%) containers and collecting tanks and (2) emptying of the waste tank (0.01%) of the chemical toilet. The PPE which have to be used for protection from the skin, eye and respiratory tract irritation potential of the biocidal product and the end-products are described as follows.

Exposure controls

Personal protective equipment:

- Chemical googles.
- Face shield.
- Substance/task appropriate gloves.
- Substance/task appropriate respirator.
- Protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure).

Organisation:

- General safety and hygiene measures:

Do not inhale gases/vapours/aerosols. Avoid contact with the skin, eyes and clothing. Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. When using, do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift. At the end of the shift the skin should be cleaned and skin-care agents applied. Gloves must be inspected regularly and prior to each use. Replace if necessary (*e.g.*, pinhole leaks).

Conclusion

During application of the end-product as disinfectant for chemical toilets, the professional worker's exposure to bronopol is estimated to be below the $AEL_{long\text{-}term}$ and therefore acceptable when RMMs for high and low hazard class chemicals are implemented and professional worker is wearing chemical googles, protective gloves, coated coverall and face mask in order to prevent any contact with the end-products. The risk of local dermal and inhalation effects during application of the final end-product is also considered to be acceptable.

C.1.3.3. Non-professional users

Systemic effects

Task/ Scenario	Tier/PPE	$AEL_{long\text{-}term}$ mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 4 application of the biocidal product concentrate to chemical toilet	1/none	0.08	1.67E-02	20.9	Yes

No unacceptable risk has been identified for the task considered.

Local effects

According to the criteria of the Regulation 1272/2008, Bronopol dilutions are proposed to be classified as:

1. 30% dilution: a severe eye irritant category 1 (H318), an irritant to skin category 2 (H315) and an irritant to respiratory tract category 3 (H335)
2. 10% dilution: a severe eye irritant category 1 (H318) and an irritant to skin category 2 (H315)
3. <10% dilution: not classified.

Therefore and as AECs can be set, quantitative and qualitative assessment of local effects will be performed in Section B.

Quantitative risk assessment

Task/ Scenario	Tier	AEC_{inhal}short-term mg/m^3	Estimated uptake mg/m^3	Acceptable (yes/no)
Scenario 4. **Application**	1	3.6	9.14E-07	Yes

This results in a mean event concentration and peak concentration which are below the AEC_{inhal}short-term of 3.6 mg/m^3. Taking this into account, the bronopol evaporation vapours exposure to is considered acceptable.

Task/ Scenario	Tier	$NOAEC_{irrit}$ % (ppm)	Estimated uptake % (ppm)	Acceptable (yes/no)
Scenario 4. **Application**	1	0.5 (5000)	10(100000)	**No**

This results in a mean event concentration and peak concentration which are above the $NOAEC_{irrit}$ of 0.5%. Taking this into account, the bronopol **dermal exposure** to is considered **not** acceptable.

Qualitative risk assessment

Primary Exposure / Non Professional use – Use of the diluted products

Hazard			Exposure							Risk
Hazard Category	**Effects in terms of C&L**	**Additional relevant hazard information**	**PT**	**Who is exposed?**	**Tasks, uses, processes**	**Potential exposure route**	**Frequency and duration of potential exposure**	**Potential degree of exposure**	**Relevant RMM&PPE**	**Conclusion on risk**
High	Eye Dam, Cat. 1 (H318)	Concentration ≥10%	2	Non Professional users	Preparation of final end-use product formulations from biocidal product Application of the final end-use product to chemical toilet. Manual application	Eyes	Equal to or less than once per week and equal to or less than few minutes per day	≥10%	Child proof closure Packaging eliminating exposure Labelling, instructions for use	Acceptable: + Low frequency + Used for short duration + Low amount used per event + Following instructions for use
Low	Skin irrit, Cat. 2 (H315)	≥ 10%				Skin	Equal to or less than one hour per day.	≥10%	Labelling, instructions for use that minimise exposure or possible health effects	
	STOT SE, Cat. 3 (H335)	≥ 30%				RT		≥ 30%		

The end-products (30% a.s. and 10% a.s.) has been allocated to:

1. the "High" hazard category according to the classification as a severe eye irritant (Eye Dam 1 - H318) and
2. the "Low" hazard category according to the classification as an irritant to skin (Skin Irrit 2 – H315) and/or an irritant to respiratory tract (STOT SE 3 – H335)

In the hazard categories proposed in the Guidance for Human Health Risk Assessment & evaluation (Volume III – Part B + C). The biocidal product is diluted to the recommended treatment concentration (30% a.s. or 10% a.s.) before use. The application task involves (1) replacing chemical toilet product (30% or 10%) containers and collecting tanks and (2) emptying of the waste tank (0.01%) of the chemical toilet. Since no PPE is expected to be used by a non-professional user the use of biocide by >100000 ppm bronopol is considered acceptable when assessed with qualitative risk assessment approach.

Use of the product in the proposed manner is considered acceptable if the RMM, which have to be used for protection from the eye irritant potential of the diluted product, are described as follows.

Exposure controls

Risk mitigation measures

- Labelling, instructions for use that minimise exposure or possible health effects.
- Childproof closure.
- Packaging eliminating risk for exposure.

Conclusion

Based on the results obtained in the risk assessment, the exposure of non-professional user results in level of exposure lower than the relevant reference values for systemic exposure but no for local dermal exposure. Therefore, exposure **must be** controlled through risk mitigation measures: labelling, instructions for use, childproof closure, packaging eliminating exposure.

C.1.3.4. Secondary (indirect) exposure as a result of use

Systemic effects

Task/ Scenario	Tier/PPE	$AEL_{short-term}$ mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 6 Use of chemical toilets	1/none	0.08	1.05E-04	0.131	Yes

Task/ Scenario	Tier/PPE	$AEL_{short-term}$ mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 7 Inhalation of volatilized residues	1/none	0.08	5.59E-11	6.99E-08	Yes
Scenario 7a Exposure to vapour formaldehyde	1/none	0.15	2.53E-07	0.000169	Yes

Task/ Scenario	Tier/PPE	AECinh mg/m³	Estimated uptake mg/m³	Estimated uptake/ AEC (%)	Acceptable (yes/no)
Scenario 7a Exposure to vapour formaldehyde	1/none	0.12	5.07E-07	0.000423	Yes
		0.12	7.30E-05	0.0608	Yes

Combined scenarios

Task/ Scenario	Tier/PPE	$AEL_{long-term}$ mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario [4, 5, 6, 7]	1/none	0.08	1.68E-02	21	Yes

No unacceptable risk has been identified for different tasks considered.

Local effects

Indirect dermal exposure to BP is possible through contact treated areas.

Residues of BNP on treated areas are predicted to be low due to the final concentration of BNP is assumed to be lower than 100 ppm.

Conclusion

Based on the results obtained in the risk assessment, the exposure of general public results in level of exposure lower than the relevant reference values for systemic exposure and local inhalation and dermal exposure. Therefore, no unacceptable risk can be identified.

C.1.3.5. Indirect exposure via food

The product is not intended to be used in places where food is kept or entrance in contact with food during its application. Therefore, no risk is derived for consumers via residues in food. In addition, in order to avoid any potential risk by its use, the following RMM is set on product's label:

- Do not use/apply directly on or near food, feed or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinks and livestock/pets.

C.1.3.6. Risk characterisation from combined exposure to several active substances or substances of concern within a biocidal product

The representative biocidal product does not contain any further active substance or substance of concern so a combined exposure is not expected.

The representative biocidal product does not contain any further active substance or substance of concern so a combined exposure is not expected C.1.3.7. Indirect exposure of animals.

The biocidal product is intended to be used indoors areas where animal presence is foreseeable. Therefore, to prevent any exposure of animals the following RMMs are included:

- Do not use/apply directly on or near food, feed or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinks and livestock/pets.

C.1.3.8. Production / formulation of active substance

Production and formulation is addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

C.1.3.9. Aggregated exposure

To be further developed once the methodology has been developed.

C.1.4 PT11

C.1.4.2. Professional uses

Systemic effects

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 3 Exposure during post-application/maintenance phase	1/none	0.08	4.60E-05	0.0574	Yes
	2/ PPE	0.08	1.91E-05	0.0238	Yes
Scenario 5 Cleaning fouled systems	1/none	0.08	8.27E-04	1.03	Yes
	2/ PPE	0.08	3.43E-04	0.429	Yes

When the exposure to diluted in-use fluid during maintenance and cleaning activities is calculated, the estimated systemic exposure of professional worker is considered acceptable without PPEs.

Due to irritant properties of biocidal product any exposure to the biocidal product must be prevented using the technical and organizational RMM adequate for high and low hazard chemicals and appropriate PPE must be used (see next chapter for details).

Local effects

According to the criteria of the Regulation 1272/2008, Bronopol dilutions are proposed to be classified as:

1. 30% dilution: a severe eye irritant category 1 (H318), an irritant to skin category 2 (H315) and an irritant to respiratory tract category 3 (H335).
2. 10% dilution: a severe eye irritant category 1 (H318) and an irritant to skin category 2 (H315).
3. <10% dilution: not classified.

Therefore and as AECs can be set, quantitative and qualitative assessment of local effects will be performed in Section B.

Quantitative risk assessment

Task/ Scenario	Tier	$NOAEC_{irrit}$ short-term % (ppm)	Estimated uptake % (ppm)	Acceptable (yes/no)
Scenario 3. **Application**	1	0.5 (5000)	0.0005 (5)	Yes

This results in a mean event concentrations and peak concentrations which are above the $NOAEC_{irrit}$ of 0.5%. Taking this into account, the bronopol dermal exposure to is considered acceptable when workers wears PPE (protective gloves and coveralls).

Qualitative risk assessment

Primary Exposure / Professional use – Use of the diluted products

Hazard			Exposure							Risk
Hazard Category	**Effects in terms of C&L**	**Additional relevant hazard information**	**PT**	**Who is exposed?**	**Tasks, uses, processes**	**Potential exposure route**	**Frequency and duration of potential exposure**	**Potential degree of exposure**	**Relevant RMM&PPE**	**Conclusion on risk**
High	Eye Dam, Cat. 1 (H318)	Concentration ≥10%	11	Professional users	Preparation of final end-use product formulations from biocidal product	Eyes	Few minutes per day or less	≥ 10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Containment as appropriate; - Segregation of the emitting process; - Effective contaminant extraction; - Good standard of general ventilation; - Minimisation of manual phases; - Regular cleaning of equipment and work area; - Avoidance of contact with contaminated tools and objects; **Organisation** - Minimise number of staff exposed; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** • Chemical goggles	Acceptable: + Minimisation of manual phases. + Low frequency + Used for short duration + Low amount used per event; + Professionals using PPE; + Professionals following instructions for use; Good standard of personal hygiene.

Low	Skin irrit, Cat. 2 (H315)	≥ 10%				Skin	More than few minutes but equal to or less than few hours per day.	≥10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Minimisation of manual phases/work tasks; - Minimisation of splashes and spills; - Avoidance of contact with contaminated tools and objects; - Regular cleaning of equipment and work area; **Organisation** - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** - Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure)	
	STOT SE, Cat. 3 (H335)	≥ 30%				RT		≥ 30%		

The end-products (30% a.s. and 10% a.s.) has been allocated to:

1. the "High" hazard category according to the classification as a severe eye irritant (Eye Dam 1 - H318) and
2. the "Low" hazard category according to the classification as an irritant to skin (Skin Irrit 2 – H315) and an irritant to respiratory tract (STOT SE 3 – H335)

In the hazard categories proposed in the Guidance for Human Health Risk Assessment & evaluation (Volume III – Part B + C). The biocidal product is diluted to the recommended treatment concentration (30% a.s.) before use. The application task involves Biocidal product dilution transferred to the sump (30% or 10%) and the post-application involves the exposure to diluted in-use fluid during maintenance and cleaning activities (0.0005%). The PPE which have to be used for protection from the skin, eye and respiratory tract irritation potential of the biocidal product and the end-products are described as follows.

Exposure controls
Personal protective equipment:

- Chemical googles.
- Face shield.
- Substance/task appropriate gloves.
- Substance/task appropriate respirator.
- Protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure).

Organisation:

- General safety and hygiene measures:

Do not inhale gases/vapours/aerosols. Avoid contact with the skin, eyes and clothing. Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. When using, do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift. At the end of the shift the skin should be cleaned and skin-care agents applied. Gloves must be inspected regularly and prior to each use. Replace if necessary (*e.g.*, pinhole leaks).

Conclusion

During application and post-application of the end-product as cooling water preservatives, the professional worker's exposure to bronopol is estimated to be below the $AEL_{long\text{-}term}$ and therefore acceptable when RMMs for high and low hazard class chemicals are implemented and professional worker is wearing chemical googles, protective gloves, coated coverall and face mask in order to prevent any contact with the end-products. The risk of local dermal and inhalation effects during application of the final end-product is also considered to be acceptable.

C.1.4.3. Non-professional users

Not applicable

C.1.4.4 Secondary (indirect) exposure as a result of use

Systemic effects

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 7 Exposure to aerosols	1/none	0.08	3.47E-3	0.096	Yes

No unacceptable risk has been identified for the tasks considered

Local effects

Indirect dermal exposure to BP is possible through contact treated areas.

Residues of BNP on treated areas are predicted to be low due to the final concentration of BNP is assumed to be lower than 5 ppm.

Conclusion

Based on the results obtained in the risk assessment, the exposure of general public results in level of exposure lower than the relevant reference values for systemic exposure and local inhalation and dermal exposure. Therefore, no unacceptable risk can be identified.

C.1.4.5. Indirect exposure via food

The product is not intended to be used in places where food is kept or entrance in contact with food during its application. Therefore, no risk is derived for consumers via residues in food. In addition, in order to avoid any potential risk by its use, the following RMM is set on product's label:

- Do not use/apply directly on or near food, feed or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinks and livestock/pets.

C.1.4.6. Risk characterisation from combined exposure to several active substances or substances of concern within a biocidal product

The representative biocidal product does not contain any further active substance or substance of concern so a combined exposure is not expected.

C.1.4.7. Indirect exposure of animals

The biocidal product is intended to be used indoors areas where animal presence is foreseeable. Therefore, to prevent any exposure of animals the following RMMs are included:

- Do not use/apply directly on or near food, feed or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinks and livestock/pets.

C.1.4.8. Production / formulation of active substance

Production and formulation is addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

C.1.4.9. Aggregated exposure

To be further developed once the methodology has been developed.

C.1.5 PT12

C.1.5.2. Professional uses

Systemic effects for primary exposure

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 2 Post-Application cleaning dispensing pumps	1/none	0.08	5.52E-01	6890	**No**
	2/ PPE	0.08	3.72E-03	4.65	Yes
Scenario 3 Post-application: process water sampling	1/none	0.08	4.60E-05	0.0574	Yes
	2/ PPE	0.08	6.20E-06	0.0077	Yes
Scenario 4 Post-application: maintenance	1/none	0.08	2.21E-03	2.76	Yes
	2/ PPE	0.08	2.98E-04	0.372	Yes

Combined scenarios

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario [2, 3]	1/none	0.08	5.52E-01	689	**No**
	2/ PPE	0.08	3.73E-03	4.66	Yes

During post-application cleaning dispensing pumps, the estimated systemic exposure of professional worker is considered acceptable when PPEs are used (protective gloves). During post-application: process water sampling and Post-application: maintenance, the estimated systemic exposure of professional worker is considered acceptable without PPEs.

Assuming one person is performing all tasks, the combined exposure was estimated. The estimated systemic exposure of professional worker is below the reference value only when protective gloves are worn.

Systemic effects for secondary exposure

Task/ Scenario	Tier/PPE	$AEL_{short-term}$ mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario 6a Post-application from papermills (vapour phase)	1/none	0.08	1.27E-06	0.0015	Yes
Scenario 6b Post-application from papermills (aerosol phase)	1/none	0.08	6.75E-04	0.84	Yes
Scenario 7 Secondary exposure to workers from papermills (contact with paper	1/none	0.08	4.2E-02	52.5	Yes

Combined scenarios

Task/ Scenario	Tier/PPE	AEL mg/kg bw/d	Estimated uptake mg/kg bw/d	Estimated uptake/ AEL (%)	Acceptable (yes/no)
Scenario [2, 3, 6a, 6b, 7]	1/none	0.08	5.54E-01	693	**No**
	2/ PPE	0.08	4.70E-03	5.88	Yes

No unacceptable risk has been identified for different tasks considered

Assuming one person could be exposed to all scenarios, the combined exposure was estimated. The total estimated systemic exposure (primary and secondary exposure) of professional worker is below the reference value only when protective gloves are worn.

Due to irritant properties of the end-product any exposure to the end-product must be prevented using the technical and organizational RMM adequate for high and low hazard chemicals and appropriate PPE must be used (see next chapter for details).

Local effects

According to the criteria of the Regulation 1272/2008, Bronopol dilutions are proposed to be classified as:

1. 30% dilution: a severe eye irritant category 1 (H318), an irritant to skin category 2 (H315) and an irritant to respiratory tract category 3 (H335)
2. 10% dilution: a severe eye irritant category 1 (H318) and an irritant to skin category 2 (H315)
3. <10% dilution: not classified.

Therefore. An a AECs can be set, quantitative and qualitative assessment of local effects will be performed in Section B.

Quantitative risk assessment

Task/ Scenario	Tier	$NOAEC_{irrit}$ % (ppm)	Estimated uptake % (ppm)	Acceptable (yes/no)
Scenario 2. Post-application	1	0.5 (5000)	030 (300000)	**No**
Scenario 3. Post-application	1	0.5 (500)	0.001 (10)	Yes

This results in a mean event concentrations and peak concentrations which are above the $NOAEC_{irrit}$ of 0.5% with the exception of scenario 3. Taking this into account, the bronopol dermal exposure to is considered acceptable when workers wears PPE (protective gloves and coveralls).

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Qualitative risk assessment

Primary Exposure / Professional use – Use of diluted product

Hazard			Exposure							Risk
Hazard Category	**Effects in terms of C&L**	**Additional relevant hazard information**	**PT**	**Who is exposed?**	**Tasks, uses, processes**	**Potential exposure route**	**Frequency and duration of potential exposure**	**Potential degree of exposure**	**Relevant RMM&PPE**	**Conclusion on risk**
High	Eye Dam, Cat. 1 (H318)	Concentration ≥10%	12	Professional users	Preparation of final end-use product formulations from biocidal product Post-application: cleaning of dispensing pumps	Eyes	Few minutes per day or less	≥ 10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Containment as appropriate; - Segregation of the emitting process; - Effective contaminant extraction; - Good standard of general ventilation; - Minimisation of manual phases; - Regular cleaning of equipment and work area; - Avoidance of contact with contaminated tools and objects; **Organisation** - Minimise number of staff exposed; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** • Chemical goggles	Acceptable: + Minimisation of manual phases. + Low frequency + Used for short duration + Low amount used per event; + Professionals using PPE; + Professionals following instructions for use; Good standard of personal hygiene.

Low	Skin irrit, Cat. 2 (H315)	≥ 10%				Skin	More than few minutes but equal to or less than few hours per day.	≥10%	**Measures to ensure well controlled exposure, such as:** **Technics** - Minimisation of manual phases/work tasks; - Minimisation of splashes and spills; - Avoidance of contact with contaminated tools and objects; - Regular cleaning of equipment and work area; **Organisation** - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene. **<u>Personal protective equipment</u>** - Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure)	
	STOT SE, Cat. 3 (H335)	≥ 30%				RT		≥ 30%		

The the end-products (30% a.s.) has been allocated to:

3. the "High" hazard category according to the classification as a severe eye irritant (Eye Dam 1 - H318) and
4. the "Low" hazard category according to the classification as an irritant to skin (Skin Irrit 2 – H315) and/or an irritant to respiratory tract (STOT SE 3 – H335)

In the hazard categories proposed in the Guidance for Human Health Risk Assessment & evaluation (Volume III – Part B + C). The biocidal product is diluted to the recommended treatment concentration (30% a.s.) before use. The post-application task involves the exposure to diluted in-use fluid during maintenance and cleaning activities (0.002%). The PPE which have to be used for protection from the skin, eye and respiratory tract irritation potential of the biocidal product and the end-products are described as follows.

Exposure controls
Personal protective equipment:

- Chemical googles.
- Face shield.
- Substance/task appropriate gloves.
- Substance/task appropriate respirator.
- Protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure).

Organisation:

- General safety and hygiene measures:

Do not inhale gases/vapours/aerosols. Avoid contact with the skin, eyes and clothing. Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. When using, do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift. At the end of the shift the skin should be cleaned and skin-care agents applied. Gloves must be inspected regularly and prior to each use. Replace if necessary (*e.g.*, pinhole leaks).

Conclusion

During post-application of the end-product as slimicide, the professional worker's exposure to bronopol is estimated to be below the $AEL_{long\text{-}term}$ and therefore acceptable when RMMs for high and low hazard class chemicals are implemented and professional worker is wearing chemical googles, protective gloves, coated coverall and face mask in order to prevent any contact with the end-products. The risk of local dermal and inhalation effects during application of the final end-product is also considered to be acceptable.

C.1.5.3. Non-professional users

Not applicable

C.1.5.4 Secondary (indirect) exposure as a result of use

Not applicable

C.1.5.5. Indirect exposure via food

For the intended use leading to a potential dietary exposure and with available scenario, indirect exposure via food and related risks were assessed.

Risk calculations are therefore presented below for the above detailed scenario.

Table 135: Dietary risk calculation – chronic and acute results

Scenario	Child exposure (mg as/kg bw/d)	Adult exposure (mg as/kg bw/d)	% ADI		% ARfD	
			Child	Adult	Child	Adult
1a	-	7.5E-03	-	9.375	-	9.375

Scenario 1a – Food packaging for PT 12

Regarding oral exposure from residues in packaging transfered into food, exposure represents 9.375% of the ADI and ARfD. No unacceptable risk is associated with indirect exposure to BNP following food contamination via residues in paper used for food packaging.

Maximum residue limits or equivalent

Estimated residues in food from the intended use were 0.45 mg a.s./kg food (see AR B.3.3.7, p. 535), thereby exceeding the default MRL of 0.01 mg/kg that applies for bronopol (according to Art 18(1)(b) Reg 396 / 2005).

C.1.5.6. Risk characterisation from combined exposure to several active substances or substances of concern within a biocidal product

The representative biocidal product does not contain any further active substance or substance of concern so a combined exposure is not expected.

C.1.5.7. Indirect exposure of animals

The biocidal product is intended to be used indoors areas where animal presence is foreseeable. Therefore, to prevent any exposure of animals the following RMMs are included:

- Do not use/apply directly on or near food, feed or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinks and livestock/pets.

C.1.5.8. Production / formulation of active substance

Production and formulation is addressed under other EU legislation (*e.g.* Directive 98/24/EC) and not repeated under Regulation 528/2012 (this principle was agreed at Biocides Technical Meeting TMI06).

C.1.5.9. Aggregated exposure

To be further developed once the methodology has been developed.

C.2 Risk characterisation for the environment

C.2.1 Atmosphere

The vapour pressure of Bronopol of 0.000051 hPa at 20°C indicates that the substance is not volatile. Further, 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 24 days (12 h sunlight).

Conclusion: It can be concluded that Bronopol will not accumulate in the atmosphere.

C.2.2 Sewage treatment plant (STP)

	PEC/PNEC$_{STP}$
PT2	
Scenario 1: Chemical toilets	2.39E-03
PT11	
Scenario 2: Open recirculating cooling systems – contin dosing - small	4.58E-04
Scenario 3: Open recirculating cooling systems – shock dosing - small	1.99E-03
Scenario 4: Closed recirculating cooling systems – Continuous dosing a) Drainage (worst case) b) With degradation	 4.89E-03 4.21E-07
PT12 Slimicide Paper Industry	
Scenario 5: typical case	5.04E-02
Scenario 6: worst case STP	2.47E-01
Scenario 8: small factories, typical case	5.05E-02
Scenario 9: small factories, worst case	2.47E-01

Conclusion: All calculated RCR values for sewage treatment plant are far below the trigger value of RCR = 1, confirming acceptable risk for this environmental compartment considering the intended biocidal applications.

C.2.3 Aquatic compartment

	PEC/PNECwater	PEC/PNECsed
PT2		
Scenario 1: Chemical toilets	2.14E-01	2.14E-01
PT11		
Scenario 2: Open recirculating cooling systems – Continuous dosing - small	4.11E-02	4.09E-02
Scenario 3: Open recirculating cooling systems – Shock dosing - small	1.78E-01	1.77E-01
Scenario 4: Closed recirculating cooling systems – Continuous dosing a) Drainage (worst case) b) With degradation	 4.40E-01 3.77E-05	 4.40E-01 3.76E-05
PT12 Slimicide Paper Industry		
Scenario 5: typical case	2.26E-01	2.25E-01
Scenario 6: worst case STP	1.11	1.10
Scenario 7: worst case DF 1000	2.36	2.35
Scenario 8: small factories, typical case	4.52	4.51
Scenario 9: small factories, worst case	22.15	22.09

Conclusion: For the intended applications calculated RCR values for surface water and sediment are below the trigger value of RCR = 1, confirming acceptable risk for these environmental compartments, except for the worst-case scenario in paper mills and for the small factories:

- According to BPC-48 "Application is only allowed in paper factories that comply with the Industrial Emission Directive 2010/75/EU where wastewater is purified in an on-site industrial sewage treatment plant including a biological treatment step in accordance to the Best Available Techniques (BAT) as prescribed in the BAT-reference document (BREF) for the production of pulp, paper and board". Hence the typical case scenario with full on-site STP would be the only realistic case. Also the worst case but with on-site STP could be possible.
- Paper factories that are exempted from the IPPC-directive must discharge to the municipal sewer. A safe use has not been demonstrated for such cases, where only a DF = 10 is achieved, but a DF of 200 is not possible (for PT12 typical case scenario, a safe use is demonstrated only when a DF = 200 is applied).

Metabolite TNM

Summary table on calculated PEC/PNEC values for TNM		
	PEC/PNECwater	**PEC/PNECsed**
PT2		
Scenario 1: Chemical toilets	0.5	0.5
PT11		
Scenario 2: Open recirculating cooling systems – Continuous dosing	0.10	0.10
Scenario 3: Open recirculating cooling systems – Shock dosing	0.42	0.42
Scenario 4: Closed recirculating cooling systems – Continuous dosing a) Drainage (worst case) b) With degradation	 a) 1.03 b) 8.88E-05	 a) 1.03 b) 8.88E-05
PT12		
Scenario 5: Slimicide Use in Paper industry, typical case scenario	0.53	0.53
Scenario 6: Worst case with STP	2.61	2.61
Scenario 8: Small paper factories typical case	10.65	10.65

Conclusion: For the intended applications calculated PEC/PNEC values for surface water and sediment for the degradation product TNM are below the trigger value of RCR = 1, confirming acceptable risk for these environmental compartments, except for the small factories discharging to the sewer and hence releasing to the municipal STP, as well as for the worst case with STP. Also a slight excendance of the trigger value results in the total drainage of closed systems when no degradation is considered (worst case).

For PT12, further supporting information should be considered:

- According to monitoring data in "[redacted]", after 24 hours the Bronopol concentration declined from 50 ppm to 27 ppm in paper mills. So almost half Bronopol is expected to disappear in the matrix paper pulp. Considering this, and that scenario for PT12 considers half-life for degradation during paper production process as degradation in paper mill + Hydrolysis (in general, according to TAB ENV 238, only abiotic degradation should be taken into account in the paper mill system, during paper making process, but if studies of sufficient quality are available showing further degradation in the paper mill system (*e.g.* biodegradation), it can be agreed on a case-by-case basis if the respective information is taken into account). In this case, as the provided study is a monitoring data and not a biodegradation study itself, it can be considered only for a qualitative assessment.
- In the sewer not only abiotic but also biotic degradation will take place, according to literature data regarding degradation in surface water. Hence, it can be expected that much less Bronopol than estimated will actually reach the municipal STP and the real PEC/PNECs ratios will be further reduced both for parent and metabolite.

- Further, in case needed, some RMMs might be applicable, such as measurement of active substance and main metabolite at the STP effluent. Some other RMMs could be applied, such as the use of collecting tanks to store wastewater and hence achieve further degradation of the metabolite (DT50 hydrolisis for TNM around 3 days at 25 ºC). Some of these RMMs might not be feasible in a municipal STP.
- Due to the unacceptable risk to surface water and sediment, further data might be required to demonstrate a safe use from paper factories releasing to a municipal STP.

Regarding the possible mixture assessment of the parent and the metabolite TNM, the mixture toxicity seems unlikely, because of the following:

- TNM may be formed mainly biotically in the STP, but it can also be formed in a chemical toilet or in the sewer where not only abiotic degradations such as hydrolysis will be taking place. In such cases, Bronopol rapidly and highly degrades to TNM and other degradation products. As all emissions are going through STP, OECD TG 314B should be considered as a basis for stating that Bronopol and its main degradation product, TNM are very unlikety to coexist. According to CAKE simulation based on OECD TG 314B, the transformation rate from Bronopol to TNM is 99.75%, summed over the whole time period of parent substance degradation.

- In case of PT2, the risk assessment has considered that all Bronopol is reaching the STP, hence, no TNM is being considered until the STP formation. In case of PT11 and 12, only abiotic degradation of the parent during the industrial process and according to the ESDs has been considered, hence no TNM is expected until the STP.

C.2.4 Terrestrial compartment

	PEC/PNECsoil	**PEC_{GW} (trigger value of 0.1 ug/l)**
PT2 Scenario 1: Chemical toilets	1.29E-02	**0.77**
PT11		
Scenario 2: Open recirculating cooling systems – Continuous dosing - small	2.48E-03 Direct release: 8.42E-03 (no drift elim.) 8.42E-05 (99% elim)	**0.15** Direct release: 0.62 (no drift elim.) 0.03 (99% elim)
Scenario 3: Open recirculating cooling systems – Shock dosing – small	1.07E-02 Direct release: 3.65E-02 (no drift elim.) 3.65E-04 (99% elim)	**0.64** Direct release: 2.68 (no drift elim.) 0.03 (99% elim)
Scenario 4: Closed recirculating cooling systems – Continuous dosing a)Drainage (worst case) b)With degradation	 2.64E-02 2.28E-06	 **1.58** 1.36E-04
PT12 Slimicide Paper Industry		
Scenario 5: typical case	3.24E-01	**19.4**
Scenario 6: worst case STP	1.59	**94.92**
Scenario 8: small factories, typical case	1.10E-01	**6.59**
Scenario 9: small factories, worst case	1.46E-01	**8.77**

Conclusion: Soil exposure is acceptable in all cases except for the worst case with STP, but it is very close to 1. There is risk to groundwater, hence FOCUS pearl 4.4.4 is applied for refinement.

C.2.5 Groundwater

The outcome of modelling with Focus Pearl Version 4.4.4 is included in section B.4. The risk in groundwater (>0.1 µg/L) must be refined by FOCUS pearl 4.4.4 for all cases but PT11 close drainage with degradation.

The FOCUS results give values above the threshold value of current quality standard of 0.1 µg/L, EU Drinking Water Directive (98/83/EC) in all cases except for PT11 open continuous system.

Bronopol is known to degrade both abiotic and biotically, the realistic amount of a.s. reaching the STP is likely to be much lower than estimated in the scenarios.

A weight of evidence approach was agreed in ENV WGIII2023 AHF (item 10). This WoE would be enough to demonstrate a safe use, based on:

- Rapid Hydrolysis of Bronopol at ambient T and pH
- Rapid degradation of Bronopol in all acute ecotoxicity studies
- Rapid degradation in the ready biodegradability tests (borderline case)
- No bronopol has been detected (below LOD) in municipal STP sludge (monitoring data), applicable for PT2 and PT11. These monitoring data published from several sources (please see section A.4.1.4) showed that no Bronopol had been detected in any environmental compartment (below LOD) including municipal sludge and even industrial STP sludge from paper factories. For instance, the LOD in the Swedish monitoring study for sludge was 12 - 24 µg/kg dwt. Bronopol could not be detected in sludge and therefore its concentration was in all cases < LOD. Still, taking the upper LOD with 24 µg/kg dwt as input parameter for Csludge, the resulting application concentrations are: Arable: 1.20E-4 kg/ha and Grass: 2.40 E-5 kg/ha. This leads to groundwater concentrations according to FOCUS Pearl 4.4.4 of 0.006 µg/L for arable and 0.001 µg/L for grass. As a result, bronopol would not pose any risk to the groundwater compartment.
- Risk to groundwater is due to the actual modelling, but very unlikely as only 1% partitions to sludge. The degradation in the primary settler will be quite important and almost no Bronopol is expected into the primary sludge.
- For PT2, and only to be used as supporting information, there is a test in urine showing rapid degradation of Bronopol in such media.
- There is an on-going OECD TG 307 study which has been required to the applicants. The pre-test OECD TG 307 results gives a worst case DT50 of around 5 days in soil at 20 ºC (9.3 days at 12 ºC). This value cannot be used for further risk assessment at product level. This is being used as supporting data at active substance level.
- Degradation of around 10% of Bronopol via hydrolysis will take place in the sewer system following equation 1 at 12 ºC and with a HRT of 1 h: $C_{sew,eff}=(C_{sew,inf})/(1 + k * HRT_{sew})$. This would apply to the uses releasing to municipal sewer.

For PT2, due to the low PECgw, 0.428, close to the threshold value of 0.1 µg/L, this WoE can be considered enough for Bronopol.

Due to the lack of information regarding degradation products in soil until OECD TG 307 study is finalized, a worst case of 100% Bronopol transformed into TNM in soil, taking into account its lower Koc (QSAR value = 10 L/kg), has been simulated, with the DT50 of 9.3 days (the pre-test show a mineralization around 50% at day 5, hence this DT50 could also be applied to degradation products) and by following TAB ENV 10 (exposure metabolites terrestrial), with the following results:

	Elocal	PEC gw	Csludge	Appl rate arable	Appl rate grass	Worst case value with FOCUS	PECgw with pre-test 307 results DT50 = 5 days (9.3 d at 12 ºC)	FOCUS DT50 refined to 9.3 d
PT2	7.33E-02	0.77	1.10E+00	5.51E-03	1.10E-03	0.428	0.05	
PT2 TNM		-	1.10E+00	5.51E-03	1.10E-03		0.29	0.000213

PT2_considering all Bronopol in sludge turns into TNM_Koc 10_DT50soil 9.3d					
PT2_9.3d_ARABLE	RUN_ID	RESULT_TEXT	SUBSTANCE	SUS	LOCATION
	776	Concentration	sus	0.000003	CHATEAUDUN
Appl rate	777	Concentration	sus	0.000133	HAMBURG
5,51E-03	778	Concentration	sus	0.000056	KREMSMUENS
	779	Concentration	sus	0.000213	OKEHAMPTON
	780	Concentration	sus	0.000002	PIACENZA
	781	Concentration	sus	0.000000	PORTO
	782	Concentration	sus	0.000000	SEVILLA
	783	Concentration	sus	0.000000	THIVA
PT2_9.3d_GRASS	RUN_ID	RESULT_TEXT	SUBSTANCE	SUS	LOCATION
	784	Concentration	sus	0.000000	CHATEAUDUN
Appl rate	785	Concentration	sus	0.000004	HAMBURG
1,10E-03	786	Concentration	sus	0.000004	JOKIOINEN
	787	Concentration	sus	0.000002	KREMSMUENS
	788	Concentration	sus	0.000020	OKEHAMPTON
	789	Concentration	sus	0.000026	PIACENZA
	790	Concentration	sus	0.000001	PORTO
	791	Concentration	sus	0.000000	SEVILLA
	792	Concentration	sus	0.000000	THIVA

Regarding PT11, there is already a safe use, hence, there is no need for additional refinements. Further, for the case of unsafe use from the close system, it might be avoided by applying TAB ENV 126:

Closed cooling system – drainage of the system and treatment as hazardous waste

Version 1 (WG-II-2017)

It was questioned if it can be assumed as refinement that the system is completely drained and the content is collected for treatment by a specialised waste water treatment company.

It was agreed that the collection of cooling liquid and disposing it off as hazardous waste is an acceptable assumption for a RMM in the case of closed cooling system in PT 11.

Regarding PT12, a similar WoE approach as for PT2 might be applied. Considering the conclusion from BPC-48 "Application is only allowed in paper factories that comply with the Industrial Emission Directive 2010/75/EU where wastewater is purified in an on-site industrial sewage treatment plant including a biological treatment step in accordance to the Best Available Techniques (BAT) as prescribed in the BAT-reference document (BREF) for the production of pulp, paper and board". Hence the typical case scenario or a worst case but both of them with full on-site STP would be the realistic case, with a DF = 200.

Paper factories that are exempted from the IPPC-directive must discharge to the municipal sewer. Regarding those small factories (<20 tn/d) releasing to municipal STP, at product authorisation level, a safe use should be demonstrated for surface water and sediment. For groundwater, the same WoE approach would be applicable as for the typical case in industrial facilities under Industrial Emission Directive and partially as for PT2.

Hence, a refinement has been considered, by applying the pre-test results from OECD TG 307 study, with a DT50 of 9.3 days at 12 ºC (the tier 1 PEC gw is 1.19) and after FOCUS pearl refinement, as a tier 2 but only as part of the WoE approach, there would be no risk to groundwater:

	Elocal	**PEC gw**	**Csludge**	**Appl rate arable**	**Appl rate grass**	**PECgw with pre-test 307 results DT50 = 5 days (9.3 d at 12ºC)**	**FOCUS results (DT50 refined to 9.3 d)**
PT12 small	2.32E-01	6.59	9.38E+00	4.69E-02	9.38E-03	0.2	<0.1
PT12 typical case	3.87E+00	19.38	2.76E+01	1.38E-01	2.76E-02	1.19	<0.1
PT12 worst case STP	1.90E+01	94.92	1.35E+02	6.76E-01	1.35E-01	5.81	<0.1

Focus example:

PT12_9.3d_ARABLE	RUN_ID	RESULT_TEXT	SUBSTANCE	SUS	LOCATION
	776	Concentration	sus	0.000001	CHATEAUDUN
Appl rate	777	Concentration	sus	0.000027	HAMBURG
1,38E-01	778	Concentration	sus	0.000011	KREMSMUENS
	779	Concentration	sus	0.000112	OKEHAMPTON
	780	Concentration	sus	0.000001	PIACENZA
	781	Concentration	sus	0.000000	PORTO
	782	Concentration	sus	0.000000	SEVILLA
	783	Concentration	sus	0.000000	THIVA
PT12_9.3d_GRASS	RUN_ID	RESULT_TEXT	SUBSTANCE	SUS	LOCATION
	784	Concentration	sus	0.000000	CHATEAUDUN
Appl rate	785	Concentration	sus	0.000000	HAMBURG
2,76E-02	786	Concentration	sus	0.000000	JOKIOINEN
	787	Concentration	sus	0.000000	KREMSMUENS
	788	Concentration	sus	0.000000	OKEHAMPTON
	789	Concentration	sus	0.000000	PIACENZA
	790	Concentration	sus	0.000000	PORTO
	791	Concentration	sus	0.000000	SEVILLA
	792	Concentration	sus	0.000000	THIVA

C.2.6 Primary and Secondary poisoning

Conclusion: Bronopol does not accumulate in biota. This is apparent from its low log Pow of 0.17 (experimental value at pH 4 (not stable at pH 7), calculated values -0.42). Based on the low partition coefficient it was not required to determine any bioconcentration factors (BCF) for Bronopol. Estimations by QSAR yielded BCFs <1 for aquatic and terrestrial ecosystems, confirming no potential for bioaccumulation.

C.2.7 Aggregated exposure (combined for relevant emission sources)

Conclusion: Assessment of aggregated exposure is not relevant.

C.3 Risk characterisation for the physico-chemical properties

The active substance has neither explosive nor oxidising or pyrophoric properties and is not highly flammable. Accordingly, no hazard is indicated from physico-chemical properties and the active substance needs does not be classified for physical or chemical hazards in accordance with Regulation (EC) 1272/2008.

However, due to the 'not low' result of the BAM Trauzl test (see A.1.3.3), 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'.

Dust explosion class St 1 (DIN EN 14034, Part 1 and 2).

C.4 Measures to protect man, animals and the environment

Measures to protect man

PT 2

General public / Consumers

Systemic exposure of Non-professional during the use of the biocidal products and the secondary, indirect exposure has been assessed to bear no risk for the general public.

For local exposure to Bronopol, no unacceptable risk for the general public has been identified either. Concerning the classification of the biocidal product, risk mitigation measures like labelling of the product including appropriate use instructions are required. Nevertheless, during application of the sanitary additive, gloves may need to be worn as instructed on the label.

Workers

Industrial and professional workers are applying the representative product and can, therefore, be exposed to the active substance via mixing and loading activities – either by loading of the biocidal product into a final product to be preserved or by the preparation of a pre-mix prior to the initial loading.

In order to ensure an acceptable risk for those scenarios describing semi-automatic or even manual activities (assuming 100% active substance in the product), PPEs such as gloves, coverall and respirator may need to be worn. Furthermore, exposure duration should be limited to a maximum of 4 min.

Application of the sanitary additive (assuming up to 10% active substance) should be conducted using protective gloves in order to cover potential systemic effects.

Moreover, with regard to local effects, technical and organisational risk mitigation measures should be in place to show that the risk is adequately controlled.

PT 11

General public / Consumers

Non-professional use and exposure of the general public arising from secondary, indirect exposures is not intended or expected and is therefore not assessed.

Workers

Industrial and professional workers are applying the representative product and can, therefore, be exposed to the active substance via mixing and loading activities – either by loading of the biocidal product into the matrix to be preserved or by the preparation of a pre-mix prior to the initial loading.

Describing semi-automatic or even manual activities (assuming 100% active substance in the product), PPEs such as gloves, coverall and respirator may need to be worn. Furthermore, a maximum exposure duration of 4 min should be ensured.

Moreover, with regard to local effects, technical and organisational risk mitigation measures should be in place to show that the risk is adequately controlled.

PT 12

General public / Consumers

Non-professional use is not intended and is therefore not assessed.

Regarding exposure scenarios for the general public arising from secondary, indirect exposures, the relevant worst-case scenario (Mouthing of treated carton (chips) by children) has been addressed. For systemic and local exposure to Bronopol, no unacceptable risk for the general public is identified.

Workers

Industrial and professional workers are applying the representative product and can, therefore, be exposed to the active substance via mixing and loading activities – either by loading of the biocidal product into the matrix to be preserved or by the preparation of a pre-mix prior to the initial loading.

In order to ensure an acceptable risk for those scenarios describing semi-automatic or even manual activities (assuming 100% active substance in the product), PPEs such as gloves, coverall and respirator may need to be worn. Furthermore, exposure duration should be limited to a maximum of 4 min.

Moreover, with regard to local effects, technical and organisational risk mitigation measures should be in place to show that the risk is adequately controlled.

Measures for the environment

PT 2

Based on the performed risk assessment, no measures are necessary to protect the environment.

However, the environmental risk assessment assumes a maximum Bronopol concentration of 10% in the sanitary product used for the disinfection of chemical toilets and a maximum target concentration of 100 ppm in the tank of a chemical toilet. For products with higher active substance concentrations and increased target concentrations, it needs to be ensured that environmental risks are adequately controlled.

PT 11

Based on the performed risk assessment, the following measures are necessary to protect the environment.

Strong reduction of emissions to soil (and ground water) is taken into account by assuming drift elimination (efficacy 99%) for all intended cooling water applications. The respective RMM can be specified as ”Cooling towers shall be equipped with eliminators that reduce drift by at least 99%.“

For closed systems, TAB ENV 126 should be considered if no safe use can be demonstrated at product level (treatment of total drainage as a hazardous waste).

Only small systems have been considered in this risk assessment (maximum blowdown of 2 m^3/h), as large systems cannot discharge to the municipal sewer.

PT 12

Based on the performed risk assessment, the following measures are necessary to protect the environment.

Waste water must be treated in accordance with the BAT. Available techniques consist of primary and secondary treatment. The first step concerns physico-chemical treatment in order to reduce the load for the second step, the second biological treatment including secondary sedimentation to remove sewage sludge. The respective details are described in following document:

Best Available Techniques (BAT). Reference Document for the Production of Pulp, Paper and Board. Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control), Publications Office of the European Union EUR 27235 EN, Luxembourg, 2015.

Furthermore, a dilution factor of at least 200 must be achieved for the STP effluent entering the receiving water body. This is not possible neither applicable for a municipal STP, in case of factories exempted from the Industrial Emissions Directive 2010/75/UE (< 20 tons/day) releasing to the sewer.

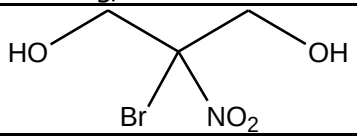
A point of special interest is the use in small factories releasing to municipal STP, as the DF of 200 is not possible to achieve and hence a safe use to surface water should be demonstrated at product level.

D. Appendices

Appendix I: List of endpoints

Chapter 1: Identity, Physical and Chemical Properties, Classification and Labelling

Active substance (ISO Name)	Bronopol
Product-type	2, 11, 12

Identity	
Chemical name (IUPAC)	2-bromo-2-nitro-1,3-propanediol
Chemical name (CA)	Bronopol
CAS No	52-51-7
EC No	200-143-0
Other substance No.	none
Minimum purity of the active substance as manufactured (g/kg or g/l)	989 g/kg
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	The identity of the impurities is confidential information and therefore not given here. There is no substance of concern.
Molecular formula	$C_3H_6BrNO_4$
Molecular mass	199.9 g/mol
Structural formula	

Physical and chemical properties	
Melting point	129 °C (decomposition at ca. 170 °C; purity 99.7%)
Boiling point	The normal boiling temperature cannot be determined. At pressures above 60 hPa temperatures decreased at constant pressures as a consequence of thermically caused changes in the test item. (purity 99.7 %)
Thermal stability / Temperature of decomposition	An exothermic process was observed starting at about 170 °C (decomposition). Onset temperature: 155 °C Peak temperature: 218 °C Energy release: 2870 J/g
Appearance	White to yellowish crystalline solid or pellets (purity 98.7–99.7%)
Relative density	D_4^{20} = 1.905 +/- 0.001 (purity 99.7%)
Surface tension	72 mN/m (20 °C; 1.0 g/L) Bronopol is not surface-active.
Vapour pressure (in Pa)	$5.1*10^{-3}$ at 20 °C 0.01 at 25 °C
Henry's law constant (Pa m3 mol -1)	$1.16*10^{-6}$ at 25 °C

<table>
<tr><td>Solubility in water</td><td>304 g/L (pH 5; 20 °C)
The determination of the water solubility at pH 7 and above is not reasonable. The test substance is not stable under these conditions.</td></tr>
<tr><td>Solubility in organic solvents</td><td>
<table>
<tr><th>Solvent</th><th>Temp [°C]</th><th>Result [g/L]</th></tr>
<tr><td rowspan="3">1-octanol</td><td>10</td><td>90.3</td></tr>
<tr><td>20</td><td>108</td></tr>
<tr><td>30</td><td>133</td></tr>
<tr><td rowspan="3">n-heptane</td><td>10</td><td><0.015</td></tr>
<tr><td>20</td><td>0.020</td></tr>
<tr><td>30</td><td>0.034</td></tr>
<tr><td rowspan="3">acetone</td><td>10</td><td>>250</td></tr>
<tr><td>20</td><td>>250</td></tr>
<tr><td>30</td><td>>250</td></tr>
<tr><td rowspan="2">methanol</td><td>20</td><td>746</td></tr>
<tr><td>30</td><td>853</td></tr>
<tr><td rowspan="2">toluene</td><td>20</td><td>1.5</td></tr>
<tr><td>30</td><td>2.5</td></tr>
</table>
</td></tr>
<tr><td>Stability in organic solvents used in biocidal products including relevant breakdown products</td><td>Not required. The active substance as manufactured does not contain organic solvents.

Bronopol is stable in organic solvents and has been commercially available for many years in liquid formulations based on propylene glycol (30% Bronopol) and dipropylene glycol monomethyl ether (40% Bronopol).</td></tr>
<tr><td>Partition coefficient (log Pow)</td><td>pH 3-4 at 10°C: −0.32
pH 3-4 at 20°C: −0.42
pH 3-4 at 30°C: −0.50</td></tr>
</table>

Dissociation constant	pKa = 9.91 ± 0.36 at 20°C Titration with alkali gave a mean pKa of 9.56 with standard deviation 0.04 and coefficient of variation of 0.4% (n=3). (The dissociation constant measured in this study was not a true pKa of Bronopol since it degrades above pH 7.0. The pKa measured was likely to be a composite pKa primarily associated with the initial decomposition product.)
UV/VIS absorption (max.)	No absorption maximum was determined between 200 nm and 800 nm Lambda max = 200 nm Specific Absorbance: 192.5±0.8 – 195.0±0.8 $l*g^{-1}*cm^{-1}$ (range of the mean values of five different samples) Molar Absorptivity: 3849.0±16.8 – 3898.8±17.0 $l*mol^{-1}*cm^{-1}$ (range of the mean values of five different samples)

Physical hazard and respective characteristics	
Explosives	Bronopol is not explosive in the sense of CLP. Label EUH044 – 'Risk of explosion if heated under confinement' should be added.
Flammable solids	Not highly flammable.
Self-reactive substances and mixtures	Bronopol is not self-reactive.
Pyrophoric solids	Bronopol is not pyrophoric.
Self-heating substances and mixtures	No self-heating was detected up to 400 °C.
Substances and mixtures which in contact with water emit flammable gases	Bronopol does not evolve flammable gases in contact with water.
Oxidising solids	Bronopol is not considered an oxidising substance.
Relative self-ignition temperature for solids	Not a readily combustible solid, no spontaneous combustion.
Dust explosion hazard	Dust explosion class St 1 (DIN EN 14034, Part 1 and 2).

Classification and proposed labelling	
with regard to physical hazards	No classification according to CLP. Label EUH044 – 'Risk of explosion if heated under confinement' should be added. Dust explosion class St 1 (DIN EN 14034, Part 1 and 2).

with regard to human health hazards	According to Council Directive 67/548/EEC: T;R25, Xn;R21, T;R23, Xi;R38, Xi;R41, Xi;R37 According to GHS: Acute Toxicity (oral): Hazard category 3 Acute Toxicity (dermal): Hazard category 4 Acute Toxicity (inhalation): Hazard category 3 Skin irritation/corrosion: Hazard category 2 - Reversible effects Serious eye damage/eye irritation: Hazard category 1 - Irreversible effects STOT SE: Hazard category 3 Hazard Statements: H301 (Toxic if swallowed) H312 (Harmful in contact with skin) H331 (Toxic if inhaled) H315 (Causes skin irritation) H318 (Causes serious eye damage) H335 (May cause respiratory irritation)
with regard to environmental hazards	According to Council Directive 67/548/EEC: R50-53 Environmental Hazard (N) According to GHS: Acute Hazards to the Aquatic Environment: Hazard category 1 Chronic Hazards to the Aquatic Environment: Hazard category 1 Hazard Statements: H400 (Very toxic to aquatic life) H410 (Very toxic to aquatic life with long lasting effects)

Chapter 2: Methods of Analysis

Analytical methods for the active substance	
Technical active substance (principle of method)	Principle of the method: RP-HPLC
Impurities in technical active substance (principle of method)	As details on the analytical methods are considered confidential information, please refer to Appendix VI for further details.

Analytical methods for residues	
Soil (principle of method and LOQ)	Principle of the method: GC-ECD LOQ= 0.05 mg/kg
Air (principle of method and LOQ)	Principle of the method: GC-ECD LOQ= 3µg/m^3

Water (principle of method and LOQ)	Principle of the method ([redacted], 2007): GC-ECD LOQ= 0.05µg/L Principle of the method ([redacted], 2007c): GC-ECD LOQ= 0.05µg/L
Body fluids and tissues (principle of method and LOQ)	Not applicable, not relevant
Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	Not applicable Bronopol and its products are not used for the treatment of food or feeding stuffs.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Not applicable Bronopol and its products are not used for the treatment of food or feeding stuffs.

Chapter 3: Impact on Human Health

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of oral absorption:	100% Based on the results of various studies, rapid and complete absorption (≥80% of applied dose) after oral exposure is concluded.
Rate and extent of dermal absorption:	43.6% 100% bronopol (powder): 10% (default value for solids) >5-30% bronopol: 10% (default value for concentrated water-based formulations) ≤5% bronopol: 50% (default value for diluted water-based formulation) Based on the results of various studies, it can be concluded that dermal absorption of 43.6% is a scientifically justified, conservative approach for Bronopol in the presence of potential penetration enhancers (diluted product). Considering the irritant nature of Bronopol 40% a dermal absorption of 43.6% can also be considered as a worst case assumption for the neat material/ undiluted product. However, for water-based mixtures, for which there is no information, default EFSA Guidance values should be applied.
Rate and extent of inhalation absorption	100% In the absence of (substance-)specific information, complete absorption (100%) is assumed for the inhalation route.
Distribution:	Bronopol is widely distributed, and highest tissue residues (beside blood) were seen in fatty tissue, skin and organs involved in excretion, *i.e.* kidney, liver, and lung.
Potential for accumulation:	No

Rate and extent of excretion:	Bronopol is rapidly excreted, mainly via urine (67-83%) whereas excretion via the faeces and exhaled air accounted for less than 10% each and played rather a minor role.
Toxicologically significant metabolite(s)	2-nitropropane-1,3-diol is the main metabolite of Bronopol in urine and plasma samples in rats.

Acute toxicity	
Rat LD50 oral	LD50 = 211 mg/kg bw (males), 193 mg/kg bw (females) Based on the results of the acute oral toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol. Bronopol is harmonised classified as Acute tox Cat. 4 H302, 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. Therefore, Bronopol is classified as Acute tox Cat. 3 H301 under Regulation (EC) No 1272/2008.
Rat LD50 dermal	LD50 = 1600 mg/kg Based on the results of the acute dermal toxicity studies available for Bronopol, a low acute toxicity potential can be concluded for Bronopol. Bronopol is harmonised classified as Acute tox Cat. 4 H312.
Rat LC50 inhalation	0.588 mg/L/4h < LC50 < 1.14 mg/L/4h Based on the results of the acute inhalation toxicity studies available for Bronopol, an acute toxicity potential can be concluded for Bronopol and the criteria for classification as Acute tox Cat. 3 H331 are met.
Skin corrosion/irritation	Bronopol causes skin irritation. Based on the results of the available animal study data and the supporting human data for Bronopol, a skin irritating potential can be concluded. Bronopol is harmonised classified as Skin irritant Cat. 2 H315.
Eye irritation	Bronopol causes eye damage. Based on the findings from eye irritation studies and an acute inhalation study available for Bronopol and in accordance with the harmonised classification Bronopol is classified as Eye damage Cat. 1 H318.

Respiratory tract irritation	Bronopol was shown to cause nasal discharge, red staining and inflammation of the eyes and staining of the head, accompanied by swelling of the head, throat and/or the forepaws at 588 mg/m^3. Moreover, Bronopol caused bloody nose and mouth breathing (at 1140 mg/m^3 only) as well as labored breathing (at 120 and 1140 mg/m^3). The observed clinical signs from the available acute inhalation toxicity studies are indicative of respiratory irritation and are addressed by the classification as STOT SE 3 H335.
Skin sensitisation (test method used and result)	Bronopol is not skin sensitising. Bronopol was found negative in two GPMTs (OECD TG 406) and an LLNA (OECD TG 429). In healthy volunteers, Bronopol did not induce skin sensitisation, while low incidences of dermal allergic responses were found in contact dermatitis patients.
Respiratory sensitisation (test method used and result)	As there are currently no appropriate tests available and there is no indication of respiratory sensitisation effects for Bronopol, respiratory sensitisation does not need to be taken into account.

Repeated dose toxicity	
Short term	
Species / target / critical effect	Short-term toxicity of Bronopol was assessed in dogs and rabbits via oral and dermal route, respectively. No treatment-related systemic toxicity has been observed in dogs and rabbits exposed to Bronopol up to the highest dose tested and key values are derived based on local effects (mild irritation) seen at the application site (skin and stomach).
Relevant oral NOAEL / LOAEL	NOAEL systemic >0.05% corresponding to 40.6 and 32.7 mg/kg bw/day for male and female dogs, respectively; local = 0.025% corresponding to 20.7 and 15.4 mg/kg bw/day for male and female dogs, respectively. LOAEL systemic = none in absence of any relevant indication for systemic toxicity; local = 0.05% corresponding to 40.6 and 32.7 mg/kg bw/day for male and female dogs, respectively.
Relevant dermal NOAEL / LOAEL	NOAEL systemic = 0.5% corresponding to 5 mg/kg bw/day for male and female rabbits; local = 0.2% corresponding to 2 mg/kg bw/day for male and female rabbits. LOAEL systemic = none in absence of any relevant indication for systemic toxicity; local = 0.5% corresponding to 5 mg/kg bw/day for male and female rabbits.
Relevant inhalation NOAEL / LOAEL	Not applicable

Sub-chronic	
Species/ target / critical effect	Sub-chronic toxicity was assessed in rats and dogs via oral route. High doses of Bronopol mainly affected water and food consumption, body weight as well as kidney weights (including signs of nephropathy) in rats.
Relevant oral NOAEL / LOAEL	NOAEL (rats) 24.3/25.5 mg/kg bw/day for males/ females and (dogs) 8 mg/kg bw/day for males/ females
Relevant dermal NOAEL / LOAEL	Not applicable
Relevant inhalation NOAEL / LOAEL	Not applicable
Long term	
Species/ target / critical effect	Long-term toxicity was assessed in rats and mice via oral route and dermal route. Main effects included reduced bw development/food/water consumption; kidney (increased weight, histopathological findings), local irritation in gastrointestinal tract.
Relevant oral NOAEL / LOAEL	NOAEL = 10 mg/kg bw/day LOAEL = 40 mg/kg bw/day
Relevant dermal NOAEL / LOAEL	NOAEL = 0.2% corresponding to 20 mg/kg bw/day
Relevant inhalation NOAEL / LOAEL	Not applicable

Genotoxicity	
In vitro / *in vivo* / Type of effect	It cannot be concluded that Bronopol does not induce genotoxicity *in vivo*. 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. Also, mutagenicity studies in mammalian cells are of very low quality and do not allow a clear result to be obtained. 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. Results from three *in vivo* test systems give no indication that Bronopol causes genotoxic effects *in vivo*, both in somatic and germ cells. However, UDS assays are known to be not sufficiently reliable due to their lack of sensitivity (only large repair areas can be detected).

Carcinogenicity	
Carcinogenicity	Carcinogenicity of Bronopol was assessed after oral and dermal exposure to rats and mice, respectively. Incidental findings were reported in both studies, which occurred in both treated and untreated animals, and showed no dose-response relationship. With no treatment-related significant increase in neoplastic findings after chronic oral and dermal exposure of rats and mice, Bronopol is considered not carcinogenic.
Species/type of tumour	Not applicable Based on the available data and in the absence of genotoxicity *in vivo*, Bronopol is considered to be non-carcinogenic.

Relevant NOAEL/LOAEL	NOAEL for systemic toxicity = 10 mg/kg bw/day (rats) and 20 mg/kg bw/day (mice) NOAEL for carcinogenicity > 160 mg/kg bw/day (rats) and > 50 mg/kg bw/day (mice) Based on the available data and in the absence of genotoxicity *in vivo*, Bronopol is considered to be non-carcinogenic.

Reproductive toxicity **Developmental toxicity**	
Species/ Developmental target / critical effect	Developmental toxicity of Bronopol was assessed in the rat and in rabbits. Pups from rabbits treated with a Bronopol dose causing maternal toxicity (including decreased body weight gain and food consumption) showed some effects clearly linked to maternal toxicity (reduced foetal body weight and incidental abnormalities of general retardation of skeletal ossification and growth), whereas rat pups were unaffected up to the maximum tolerable dose level. The key values are based on the most reliable, most conservative developmental toxicity study available for rabbits exposed to Bronopol.
Relevant developmental NOAEL	NOAEL for systemic toxicity = 10 mg/kg bw/day NOAEL for embryotoxicity = 10 mg/kg bw/day

Fertility	
Species/critical effect	Toxicity on reproduction was assessed in two 2-generation studies and a 1-generation study in the rat. Overt signs of systemic toxicity at the highest dose level were decreased body weight gain and food consumption, altered absolute and relative liver and kidney weights. No effects on the reproduction at dose levels lacking clear systemic toxicity were observed. The key values are based on the most reliable, most conservative 2-generation study available for Bronopol.
Relevant parental NOAEL	NOAEL for systemic toxicity (F0) = 10 mg/kg bw/day
Relevant offspring NOAEL	NOAEL for systemic toxicity (F1) = 50 mg/kg bw/day
Relevant fertility NOAEL	NOAEL for reproduction and fertility (F0, F1) = 50 mg/kg bw/day

Neurotoxicity	
Species/ target/critical effect	Not available

Developmental Neurotoxicity	
Species/ target/critical effect	Not available

Immunotoxicity	
Species/ target/critical effect	Not available

Developmental Immunotoxicity	
Species/ target/critical effect	Not available

Other toxicological studies	
	Not available

Medical data	
	No further data available

Summary			
	Value	**Study**	**Safety factor**
AEL	0.08 mg/kg bw/day	90-day oral toxicity study in dogs (A6.4.1_02)	100
ADI	0.08 mg/kg bw/day	90-day oral toxicity study in dogs (A6.4.1_02)	100
ARfD	0.08 mg/kg bw/day	90-day oral toxicity study in dogs (A6.4.1_02)	100
AEC_{inhal}short-term	11 mg/m³	Acute inhalation toxicity study (A6.1.3_01)	25
$NOAEC_{irrit}$	0.5%	Patch test in humans (A6_01_5-4; A6.12.6_03)	-

MRLs	
Relevant commodities	Default MRL of 0.01 mg/kg according to Art 18(1)(b) Reg. 396 / 2005

Reference value for groundwater	
According to BPR Annex VI, point 68	0.1 µg/L

Dermal absorption	
Study (*in vitro*/vivo), species tested	*In vivo*, rats
Formulation (formulation type and including concentration(s) tested, vehicle)	Bronopol was applied in acetone (considered as potential penetration enhancer) For water-based mixtures containing bronopol, for which there is no information, the default values should be used.
Dermal absorption values used in risk assessment	43.6% 100% bronopol (powder): 10% (default value for solids) >5-30% bronopol: 10% (default value for concentrated water-based formulations) ≤5% bronopol: 50% (default value for diluted water-based formulation)

Chapter 4: Fate and Behaviour in the Environment

Route and rate of degradation in water	
Hydrolysis of active substance and relevant metabolites/ degradants (DT50) (state pH and temperature)	Concentration patterns of hydrolysis products were similar in all tests. Two major hydrolysis products (> 10% a.r.) were apparent, one of which was identified by mass spectrometry as 2-bromo-2-nitroethanol. This product was transient under neutral and alkaline conditions and is not expected to be formed at significant levels under ambient temperatures in the acidic, based on the long half-life of Bronopol estimated for pH 4. The second major product was considerably more polar than Bronopol, and therefore could not be resolved or identified. It is expected that this product is a mixture including at least formaldehyde and 2-hydroxymethyl-2-nitro-1,3-propanediol (trade name: tris-hydroxymethyl-nitromethane).
pH 9	DT_{50} <2 hours at 50 °C (estimated from prelim. test) and <1 d at 25°C
Other pH: pH 7	DT_{50} = 35.28 minutes at 50 °C DT_{50} = 0.19 d at 20 ºC (indoor standard temperature) DT_{50} = 0.36 d at 12 ºC (outdoor standard temperature)
pH 4	DT_{50} = 190 days at 12 °C

Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites/ degradants	Simple first-order half-lives for midsummer sunlight days, average daylength for irradiation of 9 hours: 20 days at latitude 30-40°N 21 days at latitude 50°N The active substance was not radiolabelled and therefore metabolites could not be characterised or identified in the photolysis study. However, since half-lives for aqueous photolysis of Bronopol are relatively long compared to hydrolysis under neutral to alkaline conditions, it is expected that other processes such as hydrolysis and biodegradation would take precedence over photolysis under natural conditions. Therefore, any potential photolytic metabolites would occur at negligible concentrations only. Thus, the hydrolysis products 2-bromo-2-nitroethanol, formaldehyde and 2-hydroxymethyl-2-nitro-1,3-propanediol (trade name: tris-hydroxymethyl-nitromethane) would be expected to be formed in the aqueous environment.
Readily biodegradable (yes/no)	No
Inherent biodegradable (yes/no)	No
Biodegradation in freshwater	No study/data available, not required
Biodegradation in seawater	No study/data available, not required
Non-extractable residues	There is no indication to evolve non-extractable residues in water.
Distribution in water / sediment systems (active substance)	Bronopol shows altogether a low potential of adsorption to solid soil particles. Due to the fact that Bronopol is completely soluble in water and has a low log Koc, Bronopol is predominantly expected in the water phase.
Distribution in water / sediment systems (metabolites/ degradants)	No study/data available, not required

Route and rate of degradation in soil	
Mineralization (aerobic)	No study/data available, not required
Laboratory studies (range or median, with number of measurements, with regression coefficient)	No data available
DT50lab (20°C, aerobic):	not applicable
DT90lab (20°C, aerobic):	not applicable
DT50lab (10°C, aerobic):	not applicable
DT50lab (20°C, anaerobic):	not applicable
Degradation in the saturated zone:	Not applicable

Field studies (state location, range or median with number of measurements)	No data available, not required
DT50f:	not applicable
DT90f:	not applicable
Anaerobic degradation	No study/data available, not required
Soil photolysis	No study/data available, not required
Non-extractable residues	Bronopol shows altogether a low potential of adsorption to solid soil particles. Therefore, it is unlikely that it evolves non-extractable residues in soil.
Relevant metabolites - name and/or code, % of applied a.i. (range and maximum)	Not applicable
Soil accumulation and plateau concentration	No study available; the low log P_{ow} value of Bronopol of -0.42 indicates that there is no potential of bioaccumulation. Furthermore, due to the likely low concentration in soil, it is considered that Bronopol will not accumulate in significant quantities in soil.

Adsorption/desorption	
Ka , Kd	Sand Ka: 0.2284 – 0.8329; Kd: 4.6156 – 25.160 Loamy sand Ka: 0.7149 – 0.9477; Kd: 11.927 – 16264 Loam Ka: 1.9756 – 3.5455; Kd: 20.204 – 31.131 Clay loam Ka: 1.1879 – 1.2636; Kd: 8.8943 – 10.696
Kaoc , Kdoc	Sand Ka_{oc}: 388.3 – 1414; Kd_{oc}: 7847 – 42773 Loamy sand Ka_{oc}: 46.74 – 61.97; Kd_{oc}: 779.9 – 1063 Loam Ka_{oc}: 170.5 – 306.0; Kd_{oc}: 1744 – 2686 Clay loam Ka_{oc}: 36.82 – 41.31; Kd_{oc}: 290.8 – 349.7 Koc (geometric mean) = 136
pH dependence (yes / no) (if yes type of dependence)	Yes Adsorption of Bronopol and/or its degradation products was correlated to the soil pH. Higher adsorption was observed in alkaline soil that in acidic soul due to differences in the degradation pathway of Bronopol.

Fate and behaviour in air	
Direct photolysis in air	No study/data available
Quantum yield of direct photolysis	No study/data available

Photo-oxidative degradation in air	Latitude: 30-40°N Season: Midsummer DT_{50}: 20 days Latitude: 50°N Season: Midsummer DT_{50}: 21 days DT_{50} = 24.2 days (AOPWIN model; 12 h sunlight; conc. OH-radicals: 0.5 x 10^6 cm^{-3})
Volatilization	Not expected in regards of low Henry's Law Constant of 1.16 x 10^{-6} Pa*m^3*mol^{-1}; Bronopol is considered to be not volatile.

Reference value for groundwater	
According to BPR Annex VI, point 68	1 µg/L

Monitoring data, if available	
Soil (indicate location and type of study)	No data available
Surface water (indicate location and type of study)	No data available
Groundwater (indicate location and type of study)	No data available
Air (indicate location and type of study)	No data available

Chapter 5: Effects on Non-target Species

Toxicity data for aquatic species (most sensitive species of each group)			
Species	**Time-scale**	**Endpoint**	**Toxicity**
Fish *Lepomis macrochirus*	96 h, flow-through	Mortality; LC_{50}	11 mg a.s./L (measured conc.)
Oncorhynchus mykiss	28 d, flow-through	Growth (weight); NOEC	2.57 mg a.s./L (measured conc.)
Invertebrates *Daphnia magna*	48 h, static	Immobility; EC_{50}	1.04 mg a.s./L (mean measured conc.)
Daphnia magna	21 d, flow-through	Reproduction, growth; NOEC	0.06 mg a.s./L (mean measured conc.)
Algae (freshwater) *Scenedesmus subspicatus*	72 h, static	Growth inhibition; E_rC_{50} / NOEC /EC_{10}	0.0073/ 0.0026 / 0.0048 mg a.s./L (geomean measured conc.)
Algae (marine) *Skeletonema costatum*	72 hours	Growth inhibition; E_rC_{50} / NOE_rC	0.052 / 0.015 mg/L (mean measured conc.)
Microorganisms	2.5 hours	Respiration inhibition; EC_{50}	43 mg a.s./L

Toxicity data for aquatic species for 2-BNE ad TNM			
Species	**Time-scale**	**Endpoint**	**Toxicity**
Fish *Oncorhynchus mykiss* (2-BNE)	96 h, flow-through	Mortality; LC_{50}	3.0 mg a.s./L (measured conc.)
Oncorhynchus mykiss (TNM)	96 h, static	Mortality; LC_{50}	410 mg a.s./L (nominal conc.)
Invertebrates *Daphnia magna* (2-BNE)	48 h, flow-through	Immobility; EC_{50}	0.38 mg a.s./L (mean measured conc.)
Daphnia magna (TNM)	48 h, static	Immobility; EC_{50}	80 mg a.s./L (nominal conc.)
Algae (freshwater)	72 h, static	Growth	0.109/ 0.019 mg a.s./L (TWA

Toxicity data for aquatic species for 2-BNE ad TNM			
Species	**Time-scale**	**Endpoint**	**Toxicity**
Raphidocelis subcapitata (2-BNE)		inhibition; E_rC_{50} /E_rC_{10}	measured concentration)
Algae (freshwater) *Raphidocelis subcapitata* (TNM)	72 h, static	Growth inhibition; E_rC_{50} /E_rC_{10}	>4.5/0.572 mg/L (mean measured conc.)

Effects on earthworms or other soil non-target organisms	
Acute toxicity to *Eisenia foetida*	14d-LC_{50}: >500 mg/kg dry weight soil (synthetic OECD substrate)
Reproductive toxicity to *Eisenia foetida*	56d-NOEC: 500 mg/kg

Effects on soil micro-organisms	
Nitrogen mineralization	28-day EC_{50} = 78.1 mg/kg dry matter soil 28-day EC_{10} = 11.5 mg/kg dry matter soil
Carbon mineralization	28-day EC_{50} = 104.4 mg/kg dry matter soil 28-day EC_{10} = 10.4 mg/kg dry matter soil

Effects on terrestrial vertebrates	
Acute toxicity to mammals	Oral LD_{50} = 193 / 211 mg a.s./kg bw, female / male rats
Acute toxicity to birds	Oral LD_{50} >56 mg a.s./kg bw, *Anas platyrhynchos*
Dietary toxicity to birds	Mallard duck, 5d-LC_{50}: >10,000 ppm Bobwhite quail, 5d-LC_{50}: 4,488 ppm
Reproductive toxicity to birds	No study required if the dietary toxicity (LC_{50}) is above 2,000 mg/kg (BPR Annex II, Title 1, Point 9.4); this is true for Bronopol.

Effects on honeybees	
Acute oral toxicity	Not required, no exposure of honeybees
Acute contact toxicity	Not required, no exposure of honeybees

Effects on other beneficial arthropods	
Acute oral toxicity	Not required, no exposure of non-target arthropods
Acute contact toxicity	Not required, no exposure of non-target arthropods
Acute toxicity to …	No further studies/data available

Bioconcentration	
Bioconcentration factor (BCF)	The low log P_{ow} value of Bronopol of -0.42 indicates that there is no potential of bioconcentration. Due to the good solubility in water, it is expected that Bronopol will mainly remain in the water phase.
Depuration time (DT50)	Not applicable

Depuration time (DT90)	Not applicable
Level of metabolites (%) in organisms accounting for > 10 % of residues	Not applicable

Compartment	PNEC
Freshwater	0.00048 mg/L
Sediment	0.0018 mg/kg w.w.
STP	0.43 mg/L
Soil	0.21 mg/kg d.w.
Saltwater	0.0001 mg/L

Metabolites

Compartment	PNEC for TNM
Freshwater	0.0045 mg/L
Sediment	0.0207 mg/kg dw
STP	Not applicable
Soil	Not applicable as 0% TNM will partition to sludge (Simple treat for TNM*)

* Summary of simulated distribution in the STP (simple treat 4.0) for TNM: 0,0% to air, 85.54% to water, 14.46% degraded and 0% sludge.

Chapter 6: Other End Points

No further studies for other relevant endpoints available.

Appendix II: Human exposure calculations

PT02

PT11

PT12

Appendix III: Environmental emission (and exposure) calculations

SimpleTreat calculations for Bronopol:	
Mass of sewage solids	0.09 kg/d PE
Mass of O2 binding material in sewage (BOD)	60 gO2/d PE
Surplus sludge	0.02567 kg_{dwt}PE-1 d^{-1}
Biodegradation constant (15 ºC)	2.99 h^{-1}
Output:	Elimination in the solids liquid separator: • Volatilization 0,00 % • Via surplus sludge 1.22 % Total elimination from waste water 95.97 % Total emission via effluent 2.804 %

SimpleTreat calculations for TNM:	
Mass of sewage solids	0.0297 kg/d PE
Mass of O2 binding material in sewage (BOD)	38.2237 gO2/d PE
Surplus sludge	0.02117 kg_{dwt}PE-1 d^{-1}
Biodegradation constant (15 ºC)	0.0147 h^{-1}
Output:	Elimination in the solids liquid separator: • Volatilization 0.00 % • Via surplus sludge 0.00 % Total elimination from waste water 14.46 % Total emission via effluent 85.54 %

Bromide background levels:

The starting point is Clocal inf (mg BNP/L), then the concentration of bromide in the STP's effluent, considering all BNP degraded, 95.97% is converted into the bromide ion (molecular weight ratio = 0.4) and apply a DF = 10 from the STP to the surface water.

This is an example for PT11 open shock (worst case in PT11), releasing to the STP:

$C_{local\ inf}$ = 0.10 mg Bronopol/L
Br molecular weight = 79.9 g/mol
Considering the worst case that all Bronopol degraded in the STP converts into Bromide, 1 mol of Br released from 1 mol of Bronopol, which is degraded in the STP to a 95.97%, and considering the DF of 10 from a municipal STP:
Br released to surface water = 0.1 * 79.9/199.99 * 0.9597 / 10 = 0.0038 mg/L

	PT2	PT11 open	PT12 small close	Aggregated all PTs releasing to same STP (approximate)
$C_{local\ inf}$ (mg Bronopol/L)	0.0036	0.1	0.81	
mg Br/L (molecular weight ratio = 0.4 and 95.97% of Bronopol degraded)	0.0012	0.038	0.31	
After DF = 10	0.00012	0.0038	0.031	0.035

The background concentration of bromide in surface water is up to 0.5 mg/L according to literature presented in 2,2-dibromo-2-cyanoacetamide dossier. Therefore, the contribution of Bronopol-derived bromide to the relevant environmental compartment is far below the background concentration of bromide, even with an aggregated exposure of different product types through emission to same STP.

Appendix IV: List of terms and abbreviations

List of standard terms and abbreviations

Stand. term / Abbreviation	Explanation
A	ampere
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADME	administration distribution metabolism and excretion
AE	acid equivalent
AF	assessment factor
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50%
ALT	alanine aminotransferase (SGPT)
Ann.	Annex
AOEL	acceptable operator exposure level
AMD	automatic multiple development
ANOVA	analysis of variance
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATCC	American Type Culture Collection
BAF	bioaccumulation factor
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BPD	Biocidal Products Directive
BSAF	biota-sediment accumulation factor
BSP	bromosulfophthalein
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bacillus thuringiensis kurstaki*
Btt	*Bacillus thuringiensis tenebrionis*
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degrees Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application
cDNA	complementary DANN
CEC	cation exchange capacity
cf	confer, compare to
cfu	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
COD	chemical oxygen demand
CP	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
d	day(s)
DIS	draft international standard *(ISO)*
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days post inoculation
DRP	detailed review paper *(OECD)*

Stand. term / Abbreviation	Explanation
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen German Collection of Microorganisms and Cell Cultures
DT50(lab)	period required for 50 percent dissipation (under laboratory conditions) (define method of estimation)
DT90(field)	period required for 90 percent dissipation (under field conditions) (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
EC50	median effective concentration
ECD	electron capture detector
ED50	median effective dose
EDI	estimated daily intake
EINECS	European inventory of existing commercial substances
ELINCS	European list of notified chemical substances
e-mail	electronic mail
EMDI	estimated maximum daily intake
EN	European norm
EPMA	electron probe micro-analysis
ERL	extraneous residue limit
ESPE46/51	evaluation system for pesticides
EUSES	European Union system for the evaluation of substances
F	field
F0	parental generation
F1	filial generation, first
F2	filial generation, second
FBS	full base set
FELS	fish early-life stage
FIA	fluorescence immuno-assay
FID	flame ionisation detector

Stand. term / Abbreviation	Explanation
Fmol	fractional equivalent of the metabolite´s molecular weight compared to the active substance
FOB	functional observation battery
foc	organic carbon factor (compartment dependent)
fp	freezing point
FPD	flame photometric detector
FPLC	fast protein liquid chromatography
g	gram(s)
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GPC	gel-permeation chromatography
GPS	global positioning system
GSH	glutathione
GV	granulosevirus
h	hour(s)
H	Henry’s Law constant (calculated as a unitless value)
ha	hectare(s)
Hb	haemoglobin

Stand. term / Abbreviation	Explanation
HC5	concentration which will be harmless to at least 95 % of the species present with a given level of confidence (usually 95 %)
Hct	haematocrit
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography - mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HRGC	high resolution gas chromatography
HS	Shannon-Weaver index
Ht	haematocrit
HUSS	human and use safety standard
I	indoor
I50	inhibitory dose, 50%
IC50	median immobilisation concentration or median inhibitory concentration 1
ICM	integrated crop management
ID	ionisation detector
IEDI	international estimated daily intake
IGR	insect growth regulator
inh	inhalation
INT	2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazoliumchloride testing method
ip	intraperitoneal
IPM	integrated pest management
IR	infrared

Stand. term / Abbreviation	Explanation
ISBN	international standard book number
ISSN	international standard serial number
IUCLID	International Uniform Chemical Information Database
iv	intravenous
k (in combination)	kilo
k	rate constant for biodegradation
K	Kelvin
Ka	acid dissociation constant
Kb	base dissociation constant
Kads	adsorption constant
Kdes	apparent desorption coefficient
kg	kilogram
KH	Henry´s Law constant (in atmosphere per cubic metre per mole)
Koc	organic carbon adsorption coefficient
Kom	organic matter adsorption coefficient
Kow	octanol-water partition coefficient
Kp	solid-water partition coefficient
kPa	kilopascal(s)
l, L	litre
LAN	local area network
LASER	light amplification by stimulated emission of radiation
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC50	lethal concentration, median
LCA	life cycle analysis
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD50	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
ln	natural logarithm

Stand. term / Abbreviation	Explanation
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
log	logarithm to the base 10
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometre (micron)
MAC	maximum allowable concentration
MAK	maximum allowable concentration
MC	moisture content
MCC	minimum cidal concentration
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
µg	microgram
mg	milligram
MHC	moisture holding capacity
MIC	minimum inhibitory concentration
min	minute(s)
MKC	minimum killing concentration
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose

Stand. term / Abbreviation	Explanation
mm	millimetre
MMAD	mass median aerodynamic diameter
mo	month(s)
MOE	margin of exposure
mol	mole(s)
MOS	margin of safety
mp	melting point
MPN	most probable number method
MRE	maximum residue expected
MRL	maximum residue level or limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MT	material test
MW	molecular weight
n.a.	not applicable
n-	normal (defining isomeric configuration)
n	number of observations
NAEL	no adverse effect level
nd	not detected
NEDI	national estimated daily intake
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometre
NMR	nuclear magnetic resonance
no, n°	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported

Stand. term / Abbreviation	Explanation
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OEL	occupational exposure limit
OH	hydroxide
OJ	Official Journal
OM	organic matter content
Pa	pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PECA	predicted environmental concentration in air
PECS	predicted environmental concentration in soil
PECSW	predicted environmental concentration in surface water
PECGW	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PIC	prior informed consent
pic	phage inhibitory capacity
PIXE	proton induced X-ray emission
pKa	negative logarithm (to the base 10) of the acid dissociation constant
pKb	negative logarithm (to the base 10) of the base dissociation constant
PNEC	predicted no effect concentration (compartment to be added as subscript)
po	by mouth

Stand. term / Abbreviation	Explanation
POP	persistent organic pollutants
ppb	parts per billion (10 -9)
PPE	personal protective equipment
ppm	parts per million (10 -6)
PPP	plant protection product
ppq	parts per quadrillion (10 -24)
ppt	parts per trillion (10 -12)
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	product type
PT(CEN)	project team CEN
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QA	quality assurance
QAU	quality assurance unit
(Q)SAR	quantitative structure-activity relationship
r	correlation coefficient
r 2	coefficient of determination
RA	risk assessment
RBC	red blood cell
REI	restricted entry interval
RENI	Registry Nomenclature Information System
Rf	retardation factor
RfD	reference dose
RH	relative humidity
RL50	median residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
RRT	relative retention time
RSD	relative standard deviation
s	second
S	solubility
SAC	strong adsorption capacity
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography

Stand. term / Abbreviation	Explanation
SCAS	semi-continous activated sludge
SCTER	smallest chronic toxicity exposure ratio (TER)
SD	standard deviation
se	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
S/L	short term to long term ratio
SMEs	small and medium sized enterprises
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STER	smallest toxicity exposure ratio (TER)
STMR	supervised trials median residue
STP	sewage treatment plant
t	tonne(s) (metric ton)
t½	half-life (define method of estimation)
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TG	technical guideline, technical group
TGD	Technical guidance document
TID	thermionic detector, alkali flame detector
TDR	time domain reflectrometry

Stand. term / Abbreviation	Explanation
TER	toxicity exposure ratio
TERI	toxicity exposure ratio for initial exposure
TERST	toxicity exposure ratio following repeated exposure
TERLT	toxicity exposure ratio following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit
TNsG	technical notes for guidance
TOC	total organic carbon
Tremcard	transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TTC	2,3,5-triphenylterazoliumchloride testing method
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UR	unit risk
UV	ultraviolet
UVC	unknown or variable composition, complex reaction products
UVCB	undefined or variable composition, complex reaction products in biological material
v/v	volume ratio (volume per volume)
vis	visible

Stand. term / Abbreviation	Explanation
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
ww	wet weight
w/w	weight per weight

Stand. term / Abbreviation	Explanation
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Abbreviations of Organisations and Publications

Abbreviation	Explanation
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
BBA	German Federal Agency of Agriculture and Forestry
CA(S)	Chemical Abstracts (System)
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CEC	Commission of the European Communities
CEFIC	European Chemical Industry Council
CEN	European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CIPAC	Collaborative International Pesticides Analytical Council Ltd
CMA	Chemicals Manufacturers Association
COREPER	Comite des Representants Permanents
COST	European Co-operation in the field of Scientific and Technical Research
DG	Directorate General
DIN	German Institute for Standardisation

Abbreviation	Explanation
EC	European Commission
ECB	European Chemicals Bureau
ECCO	European Commission Co-ordination
ECDIN	Environmental Chemicals Data and Information Network of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe
ECETOC	European Chemical Industry Ecology and Toxicology Centre
EDEXIM	European Database on Export and Import of Dangerous Chemicals
EEC	European Economic Community
EHC	Environmental Health Criteria
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPAS	European Producers of Antimicrobial Substances
EPFP	European Producers of Formulated Preservatives
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organization
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive

Abbreviation	Explanation
	Operator Exposure Model
EWMP	European Wood Preservation Manufacturers
FAO	Food and Agriculture Organization of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GIFAP	Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GRIN	Germplasm Resources Information Network
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
ICBP	International Council for Bird Preservation
ICCA	International Council of Chemical Associations
ICES	International Council for the Exploration of the Seas
ILO	International Labour Organization
IMO	International Maritime Organisation
IOBC	International Organization for Biological Control of Noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
ISCO	International Soil Conservation Organization
ISO	International Organization for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JECFA FAO/WHO	Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
MITI	Ministry of International Trade and Industry, Japan
NATO	North Atlantic Treaty Organization
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Center for Toxicological Research (USA)
NGO	non-governmental organisation
NTP	National Toxicology Program (USA)
OECD	Organization for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
OPPTS	Office of Prevention, Pesticides and Toxic Substances (US EPA)
OSPAR	Oslo Paris Convention (Convention for the Protection of the Marine Environment of the North-East Atlantic)
PAN	Pesticide Action Network

Abbreviation	Explanation
RIVM	Netherlands National Institute of Public Health and Environmental Protection
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SETAC	Society of Environmental Toxicology and Chemistry
SI	Système International d'Unitès
SITC	Standard International Trade Classification

Abbreviation	Explanation
TOXLINE	Toxicology Information On-line
UBA	German Environmental Protection Agency
UN	United Nations
UNEP	United Nations Environment Programme
WFP	World Food Programme
WHO	World Health Organization
WPRS	West Palearctic Regional Section
WTO	World Trade Organization
WWF	World Wildlife Fund

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	Applicability	
						CAR/RAR	CLH
[redacted]	2000	Appendix VI	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2006	Appendix VI	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH	Y	N
[redacted]	2012	Appendix VI	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	Appendix VI	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2001	Appendix VI	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2003	Appendix VI	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2004	Appendix VI	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2005	Appendix VI	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2001	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2000a	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2002	A 1.3	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2000a	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition &	Y	N

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	Applicability	
						CAR/RAR	CLH
					Biosciences (Switzerland) GmbH		
[redacted]	2007b	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007c	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2000b	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007d	A 1.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2000	A 1.3.1 B 1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2006	A 1.3.1 B 1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2000b	A 1.3 B 1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2000	A 1.4	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	A 1.4	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2011	A 1.4	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	A 1.4	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	A 1.4	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	A 1.4	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2006	A 1.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2007a	A 1.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007b	A 1.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007c	A 1.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	1964	A5_1-01	Some properties of Bronopol, a new antimicrobial agent active against D22; J Pharm Pharmacol, 1964, 16, Suppl., 127T-130T (revised April 27, 1964); Published	No	-	Y	N
[redacted]	1978	A 2.2.1 A 3.7.3.2 A 3.9	The activity and safety of the antimicrobial agent Bronopol (2-bromo-2-nitropropan-1,3-diol); J Soc Cosmet Chem, 1978, 29, 3-24; Published	No	-	Y	N
Anonymous	1992	A 2.2.2; A 2.2.3	Bronopol (BNPD) - Bronopol-[redacted], [redacted] - A broad spectrum antibacterial agent; [redacted], Nottingham, England; Technical Bulletin, issue 6, February 1992; Unpublished	No	-	Y	N
[redacted]	2007	A5_2-01	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2003	A5_2-02	[redacted]; Unpublished	Yes	LANXESS Deutschland GmbH	Y	N
[redacted]	1973	A5_4-01	Some aspects of the mode of action of the antibacterial compound Bronopol (2-bromo-2-nitropropan-1,3-diol); J. appl. Bact. 36, 61-76; Published	No	-	Y	N
[redacted]	1988	A5_4-02	Antibacterial action of 2-bromo-2-nitropropane-1,3-diol (Bronopol); Antimicrob. Agents Chemother., Vol. 32, No. 11, 1693-1698; Published	No	-	Y	N
[redacted]	2001	A 3.2.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2000	A 3.2.2	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2003	A 3.2.3	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2000a	A 3.3	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2000b	A 3.4	[redacted]	Yes	LANXESS Deutschland GmbH, Nutrition &	Y	Y

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	Applicability	
						CAR/RAR	CLH
			[redacted]; GLP; Unpublished		Biosciences (Switzerland) GmbH		
[redacted]	2001	A 3.5	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2005	A 3.5	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	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	-	Y	Y
[redacted]	1983	A 3.3 A 3.5	Bronopol allergic contact dermatitis; Contact Dermatitis, 1983, 9:397-401; Published	No	-	Y	Y
[redacted]	1998		Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study; British Journal of Dermatology, 1998, 138:467-476; Published	No	-	Y	Y
[redacted]	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	-	Y	Y
[redacted]	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	-	Y	Y

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						CAR/RAR	CLH
[redacted]							
[redacted]	1986	A 3.5	Contact dermatitis due to Bronopol; Contact Dermatitis, 1986, 14:191-192; Published	No	-	Y	Y
[redacted]	1987	A 3.5	Kontaktallergie auf das Konservieringsmittel Bronopol; Hautarzt, 1987, 38:267-270; Published	No	-	Y	Y
[redacted]	1990	A 3.5	Contact allergy to Bronopol; Contact Dermatitis, 1990, 22:24-26; Published	No	-	Y	Y
[redacted]	2000	A 3.5	The safety of biocides for cosmetics and toiletries; Biocides Today, 2000, 13-14; Published	No	-	Y	Y
[redacted]	1973	A 3.5	Antimicrobials: Experimental contact sensitization in man; J Soc Cosmet Chem, 1973, 24:399-421; Published	No	-	Y	Y
[redacted]	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	-	Y	Y
[redacted]	2000	A 3.5	Occupational allergic contact dermatitis from both 2-bromo-2-nitropropane-1,3-diol and methylchloroisothiazolinone plus	No	-	Y	Y

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	Applicability	
						CAR/RAR	CLH
			methylisothiazolinone in spin finish; Contact Dermatitis, 2000, 43: 45; Published				
[redacted]	2004	A 3.5	Chemische Substanzen und Kontaktallergie – eine Bewertung von 244 Substanzen; Dermatologie in Beruf und Umwelt, 2004, 4:146-163; Published	No	-	Y	Y
[redacted]	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	-	Y	Y
[redacted]	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	-	Y	Y
[redacted]	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	-	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]							
[redacted]	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	-	Y	Y
[redacted]	2007	A 3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006	A 3.7.1.1	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006	A 3.7.1.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2001	A 3.7.2.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2006	A 3.7.2.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y

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						CAR/RAR	CLH
[redacted]	2007	A 3.7.2.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	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	-	Y	Y
[redacted]	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	-	Y	Y
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	-	Y	Y
[redacted]	2000a	A 3.8.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2000b	A 3.8.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2001a	A 3.8.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	2001b	A 3.8.2	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2001	A 3.8.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2001	A 3.8.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006	A 3.10.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2007	A 3.10.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	1992	not applicable	Bronopol: oral (gavage): rabbit developmental toxicity (teratogenicity) study; [redacted]; report (laboratory project) number: [redacted]; Unpublished	No	-	Y	Y
[redacted]	1995	not applicable	Bronopol: oral (gavage) rat developmental toxicity study; [redacted]; report (laboratory project) number: [redacted]; Unpublished	No	-	Y	Y

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						CAR/RAR	CLH
[redacted]	2008	A 3.10.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2003	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2012	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2000	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	1991	A 4.1.1.1	The reaction of geminal bromonitroalkanes with nucleophiles. Part 1. The decomposition of 2-bromo-2-nitropropane-1,3-diol ('Bronopol') in aqueous base; J. Chem. Soc. Perkin Trans. 2 (1991), 283-286; Published	No	-	Y	N
[redacted]	2007	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2002	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2001	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2000a	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2012	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
Anonymous	2007	A 4.1.1.2	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	1992	A 4.1.1.2	Biologischer Abbau von Bioziden - Beispiel BNPD [Biodegradation of biocides - case study BNPD]; Wasser, Abwasser 133 (1992), Nr. 10, 544-545; Published	No	-	Y	N
[redacted]	2012	A 4.1.1.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH,	Y	N

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						CAR/RAR	CLH
					LANXESS Deutschland GmbH		
[redacted]	2002	A 4.1.2.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2000	A 4.1.2.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007	A 4.1.1.1	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	2006	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2005	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2012		[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y

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						CAR/RAR	CLH
[redacted]	2000	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2006	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2012	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006a	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006b	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2002	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2006c	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	2006d	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2012	A 4.2.3.1	[redacted]; GLP ; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2000b	A 4.2.2	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2004	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2006e	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	Y
[redacted]	2007	A 4.2.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2007	A 4.2.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2007	A 4.2.4	[redacted] GLP; Unpublished	Yes	LANXESS Deutschland GmbH, Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2005	A 4.2.6	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH	Y	N
[redacted]	2002	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	N
[redacted]	1973	A 4.2.3.1	[redacted]; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	1989	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y
[redacted]	2002	A 4.2.3.1	[redacted]	Yes	Nutrition & Biosciences (Switzerland) GmbH, LANXESS Deutschland GmbH	Y	Y

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						CAR/RAR	CLH
[redacted]			[redacted]; GLP; Unpublished				
[redacted] *et al.*	2006	A 4.1.4	Results from the Swedish screening 2005. Subreport 2. Biocides; Swedish Environmental Research Institute, Stockholm; Report no. B1700, November 2006; Published	No	-	Y	N
[redacted]	2002	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2000	A 1.3 B 1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2002a	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2007	A 1.3	[redacted]; Unpublished	Yes	BASF SE	Y	
[redacted]	2003	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	2004	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	2006	not applicable	[redacted]; GLP; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2004	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1991	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2007	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2001	A 1.3 B 1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2007	A 1.3 B 1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	
[redacted]	2005	A 1.3	[redacted]; Unpublished	Yes	BASF SE	Y	
[redacted]	1992	A 1.3	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	2007a	A 1.3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	2000	A 1.3	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	2007	A 1.4	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2001	not applicable	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2006	A5.3.1_01	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2007	A5.3.1_02	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2003	B5.10.2_01	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2006	B5.10.2_02	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1997	B5.10.2_03	[redacted]; Unpublished	Yes	BASF SE	Y	N
Anonymous	1996a	B5.10.2_04	[redacted]; Unpublished	Yes	BASF SE	Y	N
Anonymous	1996b	B5.10.2_05	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1988	Summary (1.2) A 2	Antimicrobial Action of 2-Bromo-2-Nitropropane-1,3-Diol (Bronopol); Antimicrobial Agents and Chemotherapy (1988), Vol 32, No 11, pp 1693-1698; Published	No	Public	Y	N
[redacted]	1992	A 3.2.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	2000	A 3.2.2	[redacted]: Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1986	A 3.2.3 A 3.4	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1987	A 3.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1996	A 3.4	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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						CAR/RAR	CLH
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1974	A 3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1987	A 3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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[redacted]	1993	A 3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1973	A 3.7.1.2	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1973	A 3.7.2.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1973	A 3.7.2.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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						CAR/RAR	CLH
[redacted]	1998	A 3.8.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1974	A 3.8.2	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.8.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1976	A 3.7.3.1 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.7.3.1 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1985	A 3.7.3.1 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1998	A 3.7.3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.7.3.1 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1985	A 3.7.3.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1975	A 3.7.3.2	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.7.3.2 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1992	A 3.7.3.2 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1998	A 3.7.3.2 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1973	A 3.7.3.2 A 3.9	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1991	A 3.10.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1995	A 3.10.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1973	A 3.10.2	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1991	A 3.10.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1993	A 3.10.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1987	A 3.10.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1985	A 3.9 A 3.10.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	1973	A 3.10.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.10.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1986	A 3.16	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1986	A 3.16	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1973	A 3.16	[redacted]; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1978	A5_1-02	The activity and safety of the antimicrobial agent Bronopol (2-bromo-2-nitropropan-1,3-diol); J Soc Cosmet Chem 29: 3 - 24; Published	No	Public	Y	N
[redacted]	1983	A 3.3 A 3.5	Bronopol allergic contact dermatitis; Contact Dermatitis 9: 397-401; Published	No	Public	Y	Y
[redacted]	1983	A 3.5	Allergic contact dermatitis to 2-bromo-2-nitropropane-1,3-diol; Am Acad Dermatol 8: 157-170; Published	No	Public	Y	Y
[redacted]	1986	A 3.5	Reactions to Quaternium 15, Bronopol and Germall 115 in a standard series; Contact Dermatitis 14: 271-274; Published	No	Public	Y	Y
[redacted]	1990	A 3.5	Contact allergy to Bronopol; Contact Dermatitis 22: 24-26; Published	No	Public	Y	Y
[redacted]	1987	A 3.5	Kontaktallergie auf das Konservierungsmittel Bronopol. (German Report); Hautarzt 38: 267-270; Published	No	Public	Y	Y
[redacted]	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	Y	Y
[redacted]	1997	A 3.5	Patch testing Bronopol; Cosmetics & Toiletries magazine 112: 67-73; Published	No	Public	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1984	A 3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1984	A 3.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1977	A 3.5	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1992	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	1999	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	1994	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	1992	A 4.1.1.3.1 A 4.1.1.3.6.1 A 4.1.2.1	[redacted]; GLP; Published	Yes	BASF SE	Y	N
[redacted]	2005	A 4.1.1.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A7.4.1.1_02	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1981	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1998	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1994	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1998	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1998	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	2002	A 4.2.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1996	A 4.2.2	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1996	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1992	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	2007	A 4.2.4	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	2007	A 4.2.4	[redacted]; GLP; Unpublished		BASF SE	Y	N
[redacted]	2006	A 4.2.4	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	1986	A 4.2.4	The toxicity of a number of different bactericides to Clavibacter michiganense subsp. Michiganense (Smith 1910) Jensen 1934 comb. nov. [basonym Corynebacterium michiganenese (AL)] and to the tomato plant, Lycopersicon esculentum]; J. Applied Bacteriol. 61: 427-436; Published	No	Public	Y	N
[redacted]	1979	A 4.2.4	Phytotoxicity of fungicides and bactericides in orchid culture media; Amer. J. Bit. 66(7): 825-835; Published	No	Public	Y	N
[redacted]	1984	A 4.2.6	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A 4.2.6	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A 4.2.6	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A 4.2.6	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	1984	A 4.2.6	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2012	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	Y
[redacted]	2012	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	Y
[redacted]	2012	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	Y
[redacted]	2012	A 4.1.1.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	N
[redacted]	2012	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	N
[redacted]	2012	A 4.1.1.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	1989	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	Y
[redacted]	2002	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	Y
[redacted]	2002	A 4.1.1.2	[redacted]; GLP; Unpublished	Yes	BASF SE, Dow Benelux B.V., LANXESS Deutschland GmbH	Y	N
[redacted]	1995	B 3	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1994	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1997	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1997	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1997	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1995	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	1994	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	2003	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1996	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1996	A5.3.1_03	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1998a	B5.10.2_11	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1998b	B5.10.2_12	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1995	B5.10.2_13	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1997	B5.10.2_15	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A 4.1.1.3.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2015	Appendix VI	[redacted]; GLP; Unpublished	Yes	BASF	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2015	Appendix VI	[redacted]; GLP; Unpublished	Yes	BASF	Y	N
[redacted]	2015	Appendix VI	[redacted]; Non-GLP, Unpublished	Yes	BASF	Y	N
[redacted]	2015	Appendix VI	[redacted]; Non-GLP, Unpublished	Yes	BASF	Y	N
[redacted]	2014	Appendix VI	[redacted]; Non-GLP, Unpublished	Yes	BASF	Y	N
[redacted]	2014	Appendix VI	[redacted]; Non GLP, Unpublished	Yes	BASF	Y	N
[redacted]	2006	A 1.3 B 1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1996	B.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2007b	B 1	[redacted]; Unpublished	Yes	BASF SE	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2004	B.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	2004	B.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1985	B.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2007	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	N
[redacted]	2001	A 1.4	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1996	not applicable	[redacted]; Unpublished	Yes	BASF SE	N (not referenced in CAR)	N
[redacted]	1992	A 3.2.1	[redacted]; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1976	A 3.5	[redacted]; Unpublished	Yes	BASF SE	Y	Y
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	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1985		The stability of bronopol dissolved in tap water. [redacted]; Published	No	Public	Y	N
[redacted]	1991	A 4.1.1.1	The reaction of geminal bromonitroalkanes with nucleophiles. Part 1. The decomposition of 2-bromo-2-nitropropane-1,3-diol (Bronopol) in aqueous base; J. Chem. Soc. Perkin Trans. 2: 283-286; Published	No	Public	Y	N
[redacted]	1996	A 4.1.1.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	2006	A 4.1.1.3.6.1	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1984	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1984	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1996	A 4.2.2	[redacted]; Unpublished	Yes	BASF SE	Y	N
[redacted]	1994	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	1998	A 4.2.3.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	1987	A 3.2.1	[redacted]; GLP; Unpublished	Yes	BASF SE	Y	Y
[redacted]	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	Y	Y
[redacted]	1977	A 3.3	North American Group results; Contact Dermatitis 3: 208 - 209; Published	No	Public	Y	Y
[redacted]	2014	B 3	An unusual case of xylophagia (paper-eating); Ind Psychiatry J. 2014 Jan-Jun; 23(1): 65–67; Published	No	Public	Y	N
[redacted]	2015	B 3	Paper eating: An unusual obsessive-compulsive disorder dimension; Ind Psychiatry J. 2015 Jul-Dec; 24(2): 189–191; Published	No	Public	Y	N
[redacted]	2015	A 1.3.1	not identified; not identified; not identified; unknown; unknown	unknown	unknown	Y	N
[redacted]	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	Y	Y

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	Applicability	
						CAR/RAR	CLH
[redacted]	2020	A 1.3.1	[redacted]; GLP; Unpublished	Yes	BASF	Y	Y
[redacted]	2019	A 1.4	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH	Y	N
[redacted]	2021	A 1.3	[redacted]; GLP: Unpublished	Yes	BASF	Y	
[redacted]	2021	A 1.3	[redacted]; GLP; Unpublished	Yes	BASF	Y	
[redacted]	2020a	B5.10.2_06	[redacted]; Unpublished	Yes	BASF	Y	N
[redacted]	2020d	B5.10.2_14	[redacted]; Unpublished	Yes	BASF	Y	N
[redacted]	2020e	B5.10.2_16	[redacted]; Unpublished	Yes	BASF	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2022	A 4.1.1.2	[redacted]; GLP; Unpublihsed	Yes	BASF, LANXESS Deutschland GmbH, Microbial Control (Switzerland) GmbH	Y	N
[redacted]	2021	A7.5.2.1_01	[redacted]; GLP; Unpublished	Yes	BASF	Y	N
[redacted]	2022	A 4.3	[redacted]; GLP; Unpublished	Yes	BASF, LANXESS Deutschland GmbH, Microbial Control (Switzerland) GmbH	Y	N
[redacted]	2022	A 4.3	[redacted]; GLP; Unpublished	Yes	BASF, LANXESS Deutschland GmbH, Microbial Control (Switzerland) GmbH	Y	N
[redacted]	2001	B 1	Relative density; not identified; not identified; Unknown; Unknown	unknown	unknown	Y	N
[redacted]	2018	not applicable	[redacted]; Unpublished	Yes	Dow Benelux B.V.	Y	N
[redacted]	2020a	Appendix VI	[redacted]; GLP; Unpublished	Yes	LANXESS Deutschland GmbH	Y	N
[redacted]	2020b	Appendix VI	[redacted]	Yes	LANXESS Deutschland GmbH XS	Y	N

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	Applicability	
						CAR/RAR	CLH
			[redacted]; GLP; Unpublished				
[redacted]	2001	A.4.1.1.1.	Toxicity profile of labile preservative bronopol in water: The role of more persistent and toxic transformation products; Environmental Pollution 159(2): 609-615, ISSN 0269-7491; Published	No	Public	Y	N
[redacted]	2002	A.4.1.1.1.	Determination of bronopol and its degradation products by HPLC; J Pharm Biomed Anal. 29(1-2): 387-92. doi: 10.1016/s0731-7085(02)00078-x. PMID: 12062701; Published	No	Public	Y	N
[redacted]	2000	A.4.1.1.1.	Analysis of the stability of the preservative, bronopol, and identification of its decomposition products; Journal of the Society of Cosmetic Chemists 51: 332-333; Published	No	Public	Y	N
[redacted]	2008	A.4.1.1.1.	The Release of Formaldehyde upon Decomposition of 2-Bromo-2-nitropropan-1, 3-diol (Bronopol); Journal of Health Science 54(4): 488-492; Published	No	Public	Y	N
[redacted]	2007	A.6.8.2.	Comparative efficacy of Pycezes (bronopol) in controlling mortality of brown trout *Salmo trutta* eggs; Aquaculture Research, 38, 618-624; Published	No	Public	Y	N
[redacted]	2005	A.6.2. A.6.4.2.2.3.	Screening for androgen receptor activities in 253 industrial chemicals by *in vitro* reporter gene assays using AR-EcoScreenTM cells; Toxicol *in vitro* 19:831-842; Published	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2018	A.6.2. A.6.4. A.6.5.	*In vitro* nuclear receptor inhibition and cytotoxicity of hydraulic fracturing chemicals and their binary mixtures; Chemosphere 198:565-573; Published	No	Public	Y	N
[redacted]	2002	A.6.8.2.	Efficacy of bronopol against infection of rainbow trout (Oncorhynchus mykiss) with the fungus Saprolegnia species. Vet Rec. 2;151(18):539-41; Published	No	Public	Y	N
[redacted]	2008	A.6.3.1.3.	(Q)SARs for predicting effects relating to reproductive toxicity; QSAR & Combinational Science, 27:91-100; Published	No	Public	Y	N
CV	2012	A.6.	OECD Conceptual Framework for Endocrine Disruptors; Published	No	Public	Y	N
ECHA and EFSA, JRC, [redacted]	2018	A.6.	Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009; EFSA Journal 16(6):5311-135 pp; Published	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
US Environmental Protection Agency		A.6.	Endocrine Disruption Screening Program for the 21st Century (EDSP21)			Y	N
[redacted]	2016	A.6.4.3.2.1.	Tiered high-throughput screening approach to identify thyroperoxidase inhibitors within the ToxCast Phase I and II chemical libraries; Toxicol Sci 151(1):160-180; Published	No	Public	Y	N
[redacted]	2016	A.6.4.4.2.1.	High-throughput screening of chemical effects on steroidogenesis using H295R human adrenocortical carcinoma cells; Toxicol Sci 150(2):323-332; Published	No	Public	Y	N
[redacted]	2015	A.6.4.1.2.3. A.6.4.2.2.3.	Endocrine-Disrupting Activity of Hydraulic Fracturing Chemicals and Adverse Health Outcomes After Prenatal Exposure in Male Mice; Endocrinology 156(12):4458-73; Published	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]							
[redacted]	2014	A.6.4.1.2.3. A.6.4.2.2.3.	Estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in a drilling-dense region; Endocrinology Mar;155(3):897-907; Published	No	Public	Y	N
[redacted]	2016	A.6.4.1.2.3. A.6.4.2.2.3.	Adverse reproductive and developmental health outcomes following prenatal exposure to a hydraulic fracturing chemical mixture in female C57Bl/6 mice; Endocrinology 157:3469-3481; Published	No	Public	Y	N
[redacted]	2018	A.6.4.1.2.3. A.6.4.2.2.3.	Unconventional oil and gas chemicals and wastewater-impacted water samples promote adipogenesis via PPARγ-dependent and independent mechanisms in 3T3-L1 cells; Sci Total Environ 640-641:1601-1610; Published	No	Public	Y	N
[redacted]	1997		A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data; Regulatory Toxicology and Pharmacology Vol.25:1-5; Published	No	Public	Y	N
[redacted]	2018	A.6.4.2.2.1 A.6.4.2.2.3.	Assessment of androgen receptor agonistic/antagonistic effects on 25 chemicals in household applicants by OECD *in vitro* stably transfected	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]			transcriptional activation assays; Chemosphere 191:589-596; Published				
[redacted]	2007	A.6.4.1.2.1. A.6.4.1.2.2.	Evaluation of estrogenic activity of organic biocides using ER-binding and YES assay; Food Chem Toxicol. 45(9):1558-64; Published	No	Public	Y	N
[redacted]	2008	A.6.8.2.	Effects of 2-methyl-4-isothiazolin-3-one (MT) and Bronopol against Fungal Infection Control of Ayu and Rainbow trout Eggs and the Toxicity to their Fingerlings; Aquaculture Sci. 56(4), 559-565; Published	No	Public	Y	N
[redacted]	1992		[redacted]; Unpublished	Yes	BASF, LANXESS Deutschland GmbH, Microbial Control (Switzerland) GmbH	Y	N
[redacted]	2018	A.6.8.2.	Water Contaminants Associated With Unconventional Oil and Gas Extraction Cause Immunotoxicity to Amphibian Tadpoles; Toxicol Sci. 166(1):39-50; Published	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]	2014	A.6.4.1.2.3.	Predictive endocrine testing in the 21st century using *in vitro* assays of estrogen receptor signaling responses; Environ Sci Technol 48:8706-8716; Published	No	Public	Y	N
[redacted]	2003		Quantitative, structure-activity relationship models for prediction of estrogen receptor binding affinity of structurally diverse chemicals; Environ Toxicol Chem 22:1844-1854; Published	No	Public	Y	N
[redacted]	2006		A conceptual framework for predicting toxicity of reactive chemicals: Models for soft electrophilicity; SAR QSAR in Environmental Research, 17:413-428; Published	No	Public	Y	N
[redacted]	2013	A.6.4.5.	Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays; Chem Res Toxicol 26:878-895; Published	No	Public	Y	N

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	Applicability	
						CAR/RAR	CLH
[redacted]							
US Environmental Protection Agency	1995		Reregistration Eligibility Decision (RED) on Bronopol. Case 2770	No	Public	Y	N

Appendix VI: Confidential information

This section contains all information which shall not be made publicly available, but can be shared among all applicants (*i.e.*, members of the task force).

In contrast, the confidential information which shall not be shared among the members is not included in this dossier, but submitted to the authority by each applicant individually.

This applies to the method of manufacture, company-specific tonnages and 5-batch analyses.