CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

2,2-bis(bromomethyl)propane-1,3-diol

EC Number: 221-967-7

CAS Number: 3296-90-0

Index Number: -

Contact details for dossier submitter:

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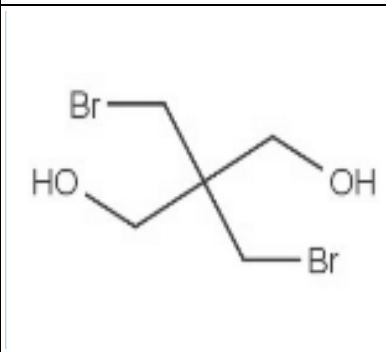
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2-bis(bromomethyl)propane-1,3-diol
Other names (usual name, trade name, abbreviation)	BMP
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	221-967-7
EC name (if available and appropriate)	2,2-bis(bromomethyl)propane-1,3-diol
CAS number (if available)	3296-90-0
Other identity code (if available)	
Molecular formula	C5H10Br2O2
Structural formula	
SMILES notation (if available)	C(CO)(CO)(CBr)CBr
Molecular weight or molecular weight range	261.94
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 98.5 %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

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Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
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Table 4: Additives (non-confidential information) if relevant for the classification of the substance

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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	-										
Dossier submitters proposal		2,2-bis(bromomethyl) propane-1,3-diol	221-967-7	3296-90-0	Muta. 1B Carc. 1B	H340 H350	GHS08 Dgr	H340 H350			
Resulting Annex VI entry if agreed by RAC and COM		2,2-bis(bromomethyl) propane-1,3-diol	221-967-7	3296-90-0	Muta. 1B Carc. 1B	H340 H350	GHS08 Dgr	H340 H350			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	**Harmonised classification proposed**	**Yes**
Carcinogenicity	**Harmonised classification proposed**	**Yes**
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has no previous harmonised classification and labelling

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

This substance is manufactured and/or imported in the European Economic Area in 100 - 1 000 tonnes per year. This substance has an industrial use resulting in manufacture of another substance (use of intermediates). It is used in polymers and in the manufacture of plastic products.

This substance can be found in products with material based on plastic (e.g. food packaging and storage, toys, mobile phones).

6 DATA SOURCES

REACH registration, ECHA dissemination site

The Chemical Safety Report

'Category approach for selected brominated flame retardants' (Danish EPA, 2016; (Wedebye et al., 2016))

Systematic literature search and relevant studies found

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Off white crystalline powder, odourless	REACH registration	
Melting/freezing point	108.5 - 109.5 °C	REACH registration	
Boiling point	ca. 270 °C	REACH registration	
Relative density	1.2 g/cm³	REACH registration	
Vapour pressure	25 deg C, mean=2x10-3 Pa	REACH registration	
Surface tension	-		
Water solubility	Water Solubility. 19.4 g/l of solution at 20.0 ± 0.5°C	REACH registration	
Partition coefficient n-octanol/water	log Pow = 1.08	REACH registration	
Flash point	-		
Flammability	-		
Explosive properties	-		
Self-ignition temperature	-		
Oxidising properties	-		
Granulometry	Particle range was between 208 um - 416 um	REACH registration	
Stability in organic solvents	-		

Property	Value	Reference	Comment (e.g. measured or estimated)
and identity of relevant degradation products			
Dissociation constant	-		
Viscosity	-		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetics Similar to OECD TG 417. Reliability score 2	After single and repeated oral administration (1, 5 and 10 days), of doses of 10 and 100 mg/kg, BMP is rapidly absorbed from the GI tract and efficiently metabolized in the liver to a glucuronide conjugate that is primarily excreted in the urine of rats. Irrespective of the dose, route or duration of exposure, less than 1% of BMP was retained in the tissues. The predominant route of BMP elimination was urinary. BMP monoglucuronide was the only metabolite present in the urine. Over 50% of the ^{14}C[BMP] dose was excreted in the bile within 6 h as glucuronide conjugate. After enterohepatic cycling, the BMP disappeared from the blood via the urine. Blood plasma concentrations of BMP after 30 min were very low. Absorption of BMP was rapid after oral administration (Cmax at 40 min). 14C equivalents were detected in the blood throughout 72 h. Both parent and BMP glucuronide were detected in the blood plasma after both	Test substance: U-^{14}C-labeled BMP Radioactively labeled BMP Purity: 97.3%. *Nonradiolabeled BMP purity: 98%* Details of study: Animals: Conventional Male F-344 rats - with indwelling jungular vein cannula - with implanted bile duct cannulas Age & weight at onset: 8-9 weeks, weighing 182-236 g Single doses: 10, 100, 150, 300 and 600 mg/kg bw oral gavage, 10 and 15 mg/kg bw for intravenous administration. Repeated dose studies: 100 mg/kg bw/day administered daily by oral gavage for 5 and 10 days Doses provided 25 to 200 µCi/kg [14C] BMP to 4 animals/study (except in the BDC study where 3 animals were used). In single dose studies, urine and feces was collected at 6 (urine only), 12, 24, 36, 48, and 72h after dose. In the repeated dose studies, urine and feces was collected at 6 (urine only), 12, and 24 h after each dose.	(Hoehle *et al.*, 2009) ECHA dossier: https://echa.europa.eu/registration-dossier/-/registered-dossier/7873/7/2/2

Method	Results	Remarks	Reference
	oral and intravenous exposure. BMP glucuronide concentration increased in the blood plasma over time. By Cmax the majority of radioactivity in blood plasma was BMP glucuronide. At oral doses of 100 mg/kg BMP and higher the urinary excretion rate, expressed as a percentage of dose, was slower than that of a dose of 10 mg/kg.	Urine cage rinse and feces were analysed for 14C content by liquid scintillation counting (LSC), HPLV-radiometric analysis and LC-MS/MS analysis. Blood and tissues (adipose, brain, cecum, cecum content, heart, intestine, intestinal contents, kidney, liver, lung, muscle, spleen, stomach, stomach content, skin, and testes) of animals were analysed. Bile samples were analysed at time points from 0.025 to 6 h, blood levels of 14C radioactivity was quantified at time points from 3 min to 48h. The blood concentration-time data were used to determine the half-life of distribution (t1/2α), terminal half-life for elimination (t1/2β) and the maximum oral bioavailability.	
Toxicokinetics study. Reliability score 1	*In vitro* glucuronidation was very low in human hepatic microsomes and intestinal microsomes as well as in human hepatocytes, and manifold lower when compared to the *in vitro* glucuronidation in hepatic microsomes and hepatocytes from other mammalian species, especially rats and mice. No other metabolites were identified. [14C]BMP and non-labeled BMP were converted into monoglucuronide by hepatic microsomal proteins or primary hepatocytes obtained from male F-344 rats. BMP glucuronide formation in hepatic F-344 rat microsomes and showed that the formation of monoglucuronide of BMP followed Michaelis-Menten kinetics. Under the same conditions of incubation as rat hepatocytes, human liver microsomes in the	Animals: Male F-344 rats Weight of animals: 200 – 325 g Microsomal fractions: Pooled microsomes were prepared from male and female F-344 rats, male B6C3F1 mice, male Golden Syrian hamster, male rhesus monkeys, and pooled human liver microsomes from 50 donors (29 males and 21 females of mix ethnicities with age ranging from 7 to 76 years). Pooled human intestinal microsomes containing equal amount of microsomes prepared from both the duodenum and jejunum of 10 donors (6 males and 4 females of mix ethnic background with age ranging from 5 to 62 years). In addition, supersomes, i.e, microsomes from insect Sf-9 cells infected with a baculovirus strain containing the cDNA of human UGT1A1, 1A3, 1A4, 1A6, 1A9 or 2B7 were also available ASSAYS Glucuronidation assay: UDP-glucuronosyltransferase (UGT)	(Rad *et al.*, 2010) This is a follow-up study of the study above.

Method	Results	Remarks	Reference
	presence of UDPGA did not convert BMP into a detectable amount of BMP glucuronide. Human intestinal microsomes converted BMP to BMP glucuronide even slower. Only one of six expressed human hepatic UGTs incubated with [14C]BMP, actively converted BMP into BMP glucuronide, at a very slow rate Less than 3% of the initial BMP concentration was converted to this metabolite over a 6-h incubation at three concentrations. The rate of BMP glucuronidation in rodent cells was 150-fold higher than in human hepatocytes. Microsomes from male B6C3F1 mice, male Golden Syrian hamster, and male Rhesus monkeys catalyzed the formation of BMP monoglucuronide, although with markedly different activities (Figure 1).	enzymes activities toward BMP, was determined in the above listed microsomes All incubations were performed in duplicate on at least three occasions. For concentration-dependent metabolism studies, incubations were conducted with [14C]BMP at final concentrations of 3.5 up to 1000 μM and rat liver microsomes. Hepatocyte incubations: Rat hepatocytes were incubated in suspension with Williams' E medium (WEM) and [14C]BMP from 2 up to 100 μM. Human hepatocytes were incubated as rats hepatocytes with [14C] for 360 min Aliquots from the rat and human hepatocyte incubations were collected at various times and analysed by HPLC Incubation with rat hepatocytes were conducted three times in duplicate for each BMP concentration The identification of conjugates of BMP from microsomal and hepatocyte incubations was done by enzymatic hydrolysis by β-glucuronidase or sulfates followed by HPLC, LC-MS and MS/MS analyses Data Analysis: The amount of glucuronide formed and glucuronidation activity were calculated, and the data for the kinetic studies were subjected to analysis based on Michaelis-Menton kinetics.	

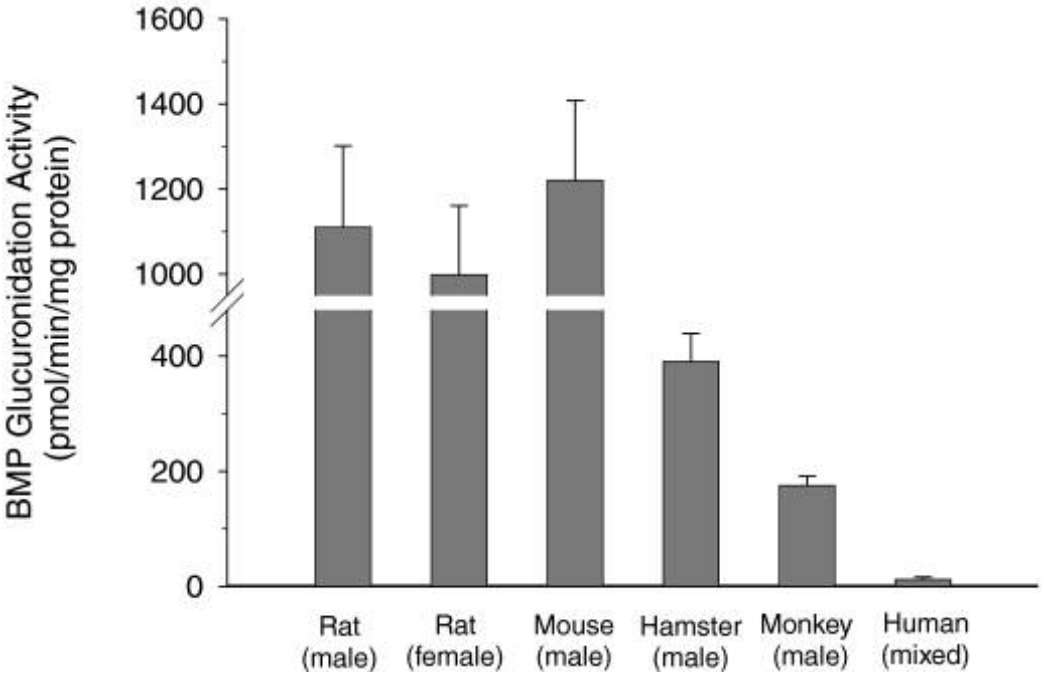

Figure 1 *Figure 6 from Rad et al., 2010. Activities of hepatic microsomes from male and female F-344 rats, male B6C3F1 mice, male Golden Syrian hamsters, male rhesus monkeys, and humans (mixed gender) for the glucuronidation of BMP. Rates of glucuronidation were determined at 50 μM BMP and are expressed as picomoles per minute per milligram of protein (mean ± S.D. of at least three independent experiments). Adopted from: (Rad et al., 2010)*

Table 10. Percentage BMP recovered in tissues and excreta in rats, Table 1 from *(Hoehle et al., 2009).*

Percentage of dose recovered from tissues and excreta after oral administration of [^{14}C]BMP (100 mg/kg) for 1, 5, or 10 daily administrations to male F-344 rats

Data are mean ± S.D.

	Fasted (72 h[a]): 1 Administration (n = 4)	Unfasted (24 h[a])		
		1 Administration (n = 3)	5 Administrations (n = 4)	10 Administrations (n = 4)
Adipose tissues	0.13 ± 0.05	0.18 ± 0.06	0.06 ± 0.02	0.04 ± 0.01
Bladder	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Bladder urine	0.02 ± 0.02	0.60 ± 0.53	0.05 ± 0.03	0.03 ± 0.03
Blood	0.20 ± 0.02	0.34 ± 0.11	0.22 ± 0.04	0.17 ± 0.01
Brain	N.D.	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cecum	0.01 ± 0.00	0.26 ± 0.07	0.11 ± 0.04	0.03 ± 0.00
Cecum contents	0.10 ± 0.05	4.08 ± 2.18	0.99 ± 0.33	0.35 ± 0.13
Cecum rinse	0.01 ± 0.01	0.55 ± 0.47	0.04 ± 0.03	0.01 ± 0.00
Heart	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Intestine	0.05 ± 0.03	1.78 ± 0.95	0.49 ± 0.16	0.14 ± 0.05
Intestine contents	0.18 ± 0.06	7.23 ± 1.94	2.30 ± 0.24	0.99 ± 0.41
Kidneys	0.01 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Liver	0.05 ± 0.01	0.65 ± 0.40	0.15 ± 0.04	0.09 ± 0.03
Lung	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.20 ± 0.03	0.34 ± 0.29	0.27 ± 0.09	0.18 ± 0.02
Skin	0.11 ± 0.02	0.25 ± 0.06	0.12 ± 0.02	0.08 ± 0.01
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Stomach contents	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Testes	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
GI tract tissues	0.07 ± 0.03	2.05 ± 0.96	0.60 ± 0.21	0.18 ± 0.05
GI tract contents	0.29 ± 0.12	11.86 ± 3.98	3.33 ± 0.42	1.35 ± 0.43
Tissues total	0.89 ± 0.18	16.05 ± 5.72	4.63 ± 0.70	1.97 ± 0.49
Urine	58.05 ± 4.28	53.40 ± 9.43	30.39 ± 5.75	19.13 ± 6.21
Cage rinse	22.73 ± 5.20	17.32 ± 0.30	44.56 ± 3.41	58.23 ± 3.18
Feces	9.68 ± 1.93	7.86 ± 1.05	14.22 ± 3.48	14.51 ± 6.31
Excreta total	90.46 ± 1.49	78.62 ± 8.42	89.17 ± 3.37	91.88 ± 2.79
Total recovery	91.56 ± 1.67	95.01 ± 4.10	94.05 ± 3.26	94.02 ± 2.42

N.D., not determined.
[a] Time after administration.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Glucuronidation appears to be the predominant route of metabolism for BMP in rodents (Hoehle *et al.*, 2009). Rodents displayed the highest glucuronidation capacity of the five species that were tested *in vitro* (figure 1)(Rad *et al.*, 2010). Glucuronidation is the sole route of metabolism of BMP in liver microsomes or primary liver cells of rodents, Rhesus monkey and human. The rate of BMP glucuronidation in rodent cells was 150-fold higher than in human hepatocytes.

In rodents BMP is rapidly absorbed from the GI tract into the portal circulation and efficiently metabolized in the liver to a glucuronide conjugate that is primarily excreted in the urine of rats. Due to the extensive metabolism the systemic exposure of BMP after ingestion in rodents is low (< 1%, see Table 25*)*. Interestingly, the urinary excretion rate was slower at higher doses than that of a dose of 10 mg/kg.

One should note that the doses are much higher and administered over a much longer time in most of the studies included in section 10.9 Carcinogenesis.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

Not evaluated in this dossier

10.1 Skin corrosion/irritation

Not evaluated in this dossier

10.2 Serious eye damage/eye irritation

Not evaluated in this dossier

10.3 Respiratory sensitisation

Not evaluated in this dossier

10.4 Skin sensitisation

Not evaluated in this dossier

10.5 Germ cell mutagenicity

Table 11: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
OECD TG 471 Bacterial Reverse Mutation Assay GLP Reliability score 2	FR522 (2,2-Bis(bromomethyl)-1,3-propanediol Purity: 98.63 Test substance concentrations: Preliminary toxicity test: 5, 50, 500, 5000 ug/plate Mutation test: 0, 50, 150, 500, 1500, 5000 ug/plate Solvent: DMSO	Initial test: Strain TA100 +/- S9-mix from rat and hamster over a wide dose range (0-5000 ug/plate). Main test: Strains TA98, TA100, TA1535 and TA1537 Metabolic activation: S9-mix from Aroclor induced rat liver cells and S9-mix from uninduced Syrian hamster liver cells	**Conclusion:** **Positive with hamster S9** **Negative with rat S9** **Negative without S9** Clear evidence of positive mutagenic activity in strains TA1535 and TA100 in the presence of Syrian hamster S9-mix as evidenced by the fairly large dose-related increases in revertant colony numbers from the two strains which were largest with *30% S9* in the assay. The test substance showed no mutagenic activity in the absence or presence of rat S9 mix Cytotoxicity: > 5000 ug/plate	Unknown author (1996). http://echa.europa.eu/registration-dossier/-/registered-dossier/7873/7/7/2
OECD TG 471 Bacterial Reverse Mutation	FR522 (2,2-Bis(bromomethyl)-1,3-propanediol Purity: 99.5	Initial test: Strain TA100 +/- S9-mix from rat and hamster over a wide dose range (0-5000 ug/plate). Main test: Strains TA98, TA100, TA1535 and	**Conclusion:** **Positive with hamster S9** **Negative with rat S9** **Negative without S9**	Unknown author (1996). http://echa.europa.eu/registration-dossier/-/registered-

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Assay GLP Reliability score 2	(purified) Test substance concentrations: Preliminary toxicity test: 5, 50, 500, 5000 ug/plate Mutation test: 0, 50, 150, 500, 1500, 5000 ug/plate Solvent: DMSO	TA1537 Metabolic activation: S9-mix from Aroclor induced rat liver cells and S9-mix from uninduced Syrian hamster liver cells	Clear evidence of positive mutagenic activity in strains TA1535 and TA100 in the presence of Syrian hamster S9- mix as evidenced by the fairly large dose-related increases in revertant colony numbers from the two strains which were largest with *30% S9* in the assay. The test substance showed no mutagenic activity in the absence or presence of rat S9 mix Cytotoxicity: > 5000 ug/plate	dossier/7873/7/7/2
Bacterial Reverse Mutation Assay Reliability score 1	Purity: ~84% Test substance concentrations: 0, 10, 33, 100, 333, 1000, 1666, 3333 and 6666 ug/plate. Solvent: DMSO	Main test: Strains TA100 and TA98. Metabolic activation: Aroclor 1254-induced S9-mix from Sprague-Dawley rat and male Syrian hamster livers in 5, 10 and 30% concentrations.	**Conclusion:** **Positive with hamster S9** **Negative with rat S9** **Negative without S9** Positive mutagenic activity with strain TA100 in the presence of *30% hamster S9-mix* as evidenced by the fairly large dose-related increases in revertant colony numbers at conc. 0-3333 ug/plate. Experiment was repeated twice for confirmation. No mutagenic activity in strain TA98. No mutagenic activity in absence of S9-mix, nor in presence of rat liver S9-mix Cytotoxicity testing up to a maximum dose of 10 mg/plate, cytotoxicity evident at >3333 ug/plate	(Zeiger *et al.*, 1992) (National Toxicology Program, 1996)
Bacterial Reverse	Purity: 96,3%	Main test: Strains TA98, TA100, TA1535 and	**Conclusion: Negative**	(Mortelmans *et*

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Mutation Assay Reliability score 1	Test substance concentrations: Preliminary toxicity test in TA100: tested over a wide range with an upper limit of 10 mg/plate, +/- S9-mix Mutation test: 0, 10, 33, 100, 333, 1000, 3333, 10000 ug/plate S9 concentration: 10% Solvent: DMSO	TA1537 Metabolic activation: Aroclor 1254-induced S9-mix from Sprague-Dawley rat and male Syrian hamster livers in 10% concentrations.	Negative results with and without *10% rat and hamster S9-mixes* in all strains tested. Cytotoxicity testing up to a maximum dose of 10 mg/plate, cytotoxicity evident at >3333 ug/plate	*al.*, 1986) (National Toxicology Program, 1996)
In vitro mammalian chromosomal aberration test Reliability score 1	Purity: Not specified Doses tested: -S9: 400, 500, 600 ug/ml +S9: 600, 800, 1000 ug/ml Maximum dose tested in preliminary tests was 1.2 mg/ml	Cell type: Chinese hamster ovary cells (CHO-W-B1). Assay: Test carried out with/without rat liver S9 mix. S9 mix: Aroclor 1254 induced male Spraque-Dawley rat liver extract. S9-concentration used not specified. Dose selection and fixation time decision criteria: Growth inhibition test and cell cycle test were conducted to determine main test concentrations and fixation times. 100 cells were scored from each of the three highest dose groups having sufficient metaphases for analysis and from control.	**Conclusion:** **Positive with rat S9** **Negative without rat S9** The aberration test without S9 was negative up to toxic level, also after extension of fixation time to 20.5h. With S9 the aberration test was positive, partly because of breaks in the long arm of the X-chromosome. The Least effect concentration (LEC; the lowest dose to give a statistically significant increase) tested was 800 ug/ml. The increase was detected at doses that did not induce obvious toxicity (reduction of confluence). This effect also occurred at highly toxic levels.	
In vitro	Purity: Not	Cell type: Chinese hamster	**Conclusion:**	

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Sister Chromatid Exchange Assay in Mammalian Cells Reliability score 1	specified Dose selection: -S9: 16.7, 50, 167 ug/ml +S9: 800, 1000, 1200 ug/ml Maximum dose tested in preliminary tests was 1.2 mg/l	ovary cells (CHO-W-B1) Assay: Test carried out with/without rat liver S9 mix S9 mix: Aroclor 1254 induced male Spraque-Dawley rat liver extract. S9-concentration used not specified. Dose selection and criteria: Growth inhibition test were conducted to determine main test concentrations. Chemical doses: Maximum 1.2 mg/ml which reduced confluence by about 75% SCE test: 5-Bromodeoxyuridine (Brdu, 10μM) based. 50 cells/dose were scored from the three highest doses at which sufficient M2 cells were available, from a solvent control and a ‘‘weak positive’’ control (only 5-10 cells were scored from the ‘‘strong positive’’ controls).	**Equivocal in the presence of rat S9** **Negative without rat S9** No increased SCE without S9 even at doses that induced toxicity and marked cell cycle delay. Very slight increases in SCEs occured at toxic levels with S9. The top dose, 1.2 mg/ml, reduced confluence by about 75%.	
In vitro comet assay BMP induced DNA breaks and oxidative stress tests	Purity: 98% Test substance concentrations: Preliminary cell viability test: 0, 250, 500, 750, 1000, 1250, 1500 uM Mutation test:0, 5, 10, 25, 100 uM	Dilution Solvent: 100% ethanol (EtoH). Cell line: Human urothelial cells (Urotsa). Vehicle concentration: 0.5% in treated and all control cultures. Test Concentration Of BMP: 10-500μM. Cell viability test: Cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Rockville,	**Conclusion:** **Genotoxic in urothelial cell line** Comet assay showed DNA damage in Urotsa cells BMP induces oxidative stress in Urotsa cells Oxidative stress plays a major role in BMP mediated DNA damage.	(Kong *et al.*, 2011)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		MD). No. cells tested: 1 x 10^4 cells/well. Assays: Comet Assay, 1 hr exposure, intracellular ROS measurement, Western immunoblotting analysis.		
In vitro comet assay Induction of genotoxicity in UROtsa cells and hepatocytes by BMP tests Reliability score 2	[14C-labeled)] Purity: 97.3% Non-labelled BMP Purity: 98% Test substance concentration: 0, 25, 50, 100 µM	Primary hepatocytes (non-target) isolated from male SD rats. 1 and 24 hr incubation of 5 x 10^5 hepatocytes (cells/ml) with [14C-] BMP (10-250 µM). Cell line: Human urothelial cells (UROtsa cells; target cells) exposed to [14C]-BMP (10–250 M) for 1 or 24 h. The single cell gel electrophoresis assay (comet assay), was applied to assess DNA strand breaks induced by BMP in cells. UROtsa cells were assayed for Beta-glucuronidase activity. DNA binding measurements was performed on treated and untreated cells. BMP metabolites were assessed by HPLC. Glutathione assay were performed to measure the total glutathione content of UROtsa and hepatocytes.	**Conclusions:** **Positive in urothelial cell line** **Negative in primary rat hepatocytes** BMP induced strand breaks in UROtsa cells but not in hepatocytes. Significant higher levels of BMP bind to UROtsa cells DNA as compared to hepatocyte cell DNA. Rat hepatocytes converted BMP to BMP-glucuronide whereas URotsa cells did not. Basal level of Glutathione (GSH) was significantly higher in untreated UROtsa cells than in hepatocyte cells. BMP treatment decreased the basal level of GSH in hepatocytes and not in UROtsa cells. Glucuronidation seem to be a detoxification mechanism in BMP associated genotoxicity.	(Kong *et al.*, 2013)

Table 12: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mouse peripheral blood micronucleus test OECD TG 474 Reliability score 1	Purity: Not specified Doses: 0, 625, 1.250, 2.500, 5.000 and 10.000 ppm in feed equivalent to approximately: Males: 0, 100, 200, 500, 1.300 and 3.000 mg/kg bw Females: 0, 140, 300, 600, 1.200 and 2.900 mg/kg bw	Micronuclei were assessed in peripheral blood in mice given BMP in feed for 13 weeks (90-day study) Animals: Male and female B6C3F1 mice 13 weeks feed study Semi-automated image analysis system 10,000 normochromatic erythrocytes (NCEs) in 7-10 animals per group.	**Conclusion: positive in both males and females** BMP was genotoxic in both male and female mice. These increases were dose related and significant for the two highest dose groups of male mice (>= 1300 mg/kg bw) and the three highest dose groups of female mice (>= 600 mg/kg bw).	NTP Technical Report Series no. 452, 1996. Appendix E. Genetic Toxicology. (National Toxicology Program, 1996)
Mouse bone marrow micronucleus test OEDC TG 474 Reliability score 1	Purity: Not specified Study 1: Doses: 0, 100, 200, 300, 400 mg/kg. Oral gavage Study 2: Doses: 0, 150, 300, 600 mg/kg. Single intraperitoneal (ip) injection	Two bone marrow micronucleus (MN) studies were performed. Study 1, two trials: Male B6C3F1 mice Oral gavage A 3-dose protocol; sampling 24h after last dosing 2,000 polychromatic erythrocytes (PCEs) scored in each of 5 animals per dose group Study 2, one trial: Male and female B6C3F1 mice Single intraperitoneal (ip) injection 1,000 PCEs were scored per animal in 3 or 4 animals in each dose group.	**Overall conclusion: positive** Study 1 (oral gavage) was inconclusive. The first trial was negative and the second positive with significant difference at the highest dose tested. Study 2 (ip injection) was positive with a significant dose-related increase in micronucleated PCEs in females (trend test, p=0.003). In males there was a significant increase in micronucleated PCEs at the highest concentration tested only, i.e. it was not dose-related.	NTP Technical Report Series no. 452, 1996. Appendix E. Genetic Toxicology. (National Toxicology Program, 1996)
In vivo mammalian	Purity: >98.0%	Animal strain: Male	**Conclusion:**	(Wada *et al.*,

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
alkaline comet assay OECD TG 489 Reliability score 1	Dosage: 0, 300 or 600 mg/kg bw	Sprague-Dawley No. of animals: 5 animals/group Route: Oral gavage Volume administered: 10 ml/kg body weight Administration: Twice at 21 h apart and sacrificed 3 h after the second dose was administered The limit doses of BMP were determined by the DNA damage induced in a dose-range finding study Comet Assay: - Cells extraction from urinary bladder tissue - Standard alkaline comet assay protocol was applied Histopathology: Histopathology was performed om bladder samples from rats treated with 600 mg/kg body BMP (suspended in methylcellulose, MT) or 0.5% MT.	**Positive in urinary bladder** **Negative in liver** DNA damage (% tail DNA) in urinary bladder was increased in animals treated with BMP at the high dose of 600 mg/kg/day; significant ($p<0.05$). No statistical increase was seen in liver. No decrease in body weight, no abnormal necropsy findings, and no carcinogenic changes in urinary bladder was observed.	2014)

Table 13: Summary table of other data relevant for germ cell mutagenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproductive assessment by continuous breeding protocol, part of the NTP	BMP Dow Chemical Company (Midland, MI), LotNo. MM 05137-636, purity 87.3%.	BMP exposure significantly decreased the numbers of litters per pair, pups born alive per litter, and pup weight when adjusted for litter size. Crossover mating between treated and control F0 animals indicated a specific effect only on female reproductive capacity. At the highest dose, BMP caused a body weight decrease in the F0 animals of both sexes with no effect	(Treinen *et al.*, 1989) National Toxicology Program, 1996

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study Animals: CD-1 albino outbred (Swiss), Charles River Labs, Kingston NY, SPF quality, 6 w old at arrival. 20 pairs per treatment group, 40 pairs of control animals Reliability score 1	Impurities: 2,2-bis(hydroxymethyl)-l-bromo-3-hydroxy-propane (6.7%), l-bromo-3-hydroxypropane (5.5%), and 3,3-bis(bromomethyl)-oxetane (0.5%)) BMP was given in the feed at 1000, 2000 and 4000 ppm (141, 274, and 589 mg/kg bw/day). For homogeneity, BMP was premixed with ethyl ether, applied to the feed, then evaporating the solvent from the mixture. Male and female F0 mice were dosed 7 days prior to and during a 98-day cohabitation period.	on relative organ weights. Sperm concentration, motility, morphology, and estrual cyclicity were unaffected by BMP exposure. Histopathology in the F0 animals revealed specific kidney lesions in both sexes; males were more sensitive than females. The last litter born in the 98-day breeding phase was reared at day 21, and for cross-over matings continued to receive BMP to age 74 +/- 10 days and then mated to non-siblings of the same treatment group. The effect of high-dose BMP exposure on F1 fertility, body and organ weights, sperm parameters, and estrual cyclicity was the same as that for the F0 animals, with the exception of the lack of renal lesions in the F1 females. These data reveal impaired fertility in BMP-treated female mice in both generations in the absence of an effect on reproductive organ weights and estrual cyclicity. Supplementary data describe that 4000 ppm BMP significantly decreased the number of primary and growing ovarian follicles in the 20 high-dose females (see Bolon et al., 1997).	
Differential Follicle Counts CD1 Reliability score 1	See description above in Treinen et al., 1989. BMP given in feed at 141, 274 and 589 mg/kg/day. F0 parents were dosed 7 days prior to and during a 98-day cohabitation period. Cross-mating F1 offspring are dosed until 74 +/- 10 days of age.	BMP significantly decrease counts of small and growing follicles at high dose in F0 animals, and in a dose response related manner with significant decreases at mid and high dose in F1-offspring. Up to 91.9% fewer small follicles and 75.8% growing follicles were counted. In F1 cross-mating mice significant decrease in follicle numbers were evident without clear evidence for reproductive toxicity, suggesting that counting follicle numbers is a more sensitive reproductive measurement than fertility.	(Bolon *et al.*, 1997)

10.5.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro studies: In the *in vitro* assays, three Ames tests are included, two giving clear concentration-related positive results in the presence of 30% Syrian hamster liver S9-mix (National Toxicology, 1996);(Zeiger *et al.*, 1992)) and one with negative result where the S9-mix concentrations were limited to 10% (Mortelmans *et al.*, 1986). In summary, positive findings were obtained when using high concentrations of hamster S9-mix (30%). No mutagenic activity was detected when using rat liver S9-mix, or low concentrations of hamster S9-mix for metabolic activation.

Moreover the OECD TG 473 Chinese hamster ovary chromosome aberration test was positive in the presence of low concentrations of rat liver S9-mix and scoring of only 100 cells per sample (Galloway *et al.*, 1987). The same authors also conducted a Sister chromatid exchange assay that was negative (Galloway *et al.*, 1987).

Through a systematic literature search, several *in vitro* Comet studies measuring DNA damage were found. All comet assays were performed with BMP of high purity (98 %). One was conducted in urothelial cells demonstrating that BMP induces DNA strand breaks and oxidative base damage in these cells (Kong *et al.*, 2011). Another *in vitro* comet study studied both a urothelial cell line and primary hepatocytes from male SD rats, repeating the positive results in the urothelial cells but no genotoxic effect was evident in the hepatocytes (Kong *et al.*, 2013). The authors suggested that the negative result in primary rat hepatocytes was due to detoxification by glucuronidation in the hepatocytes (Kong *et al.*, 2013). This is consistent with studies of metabolism of BMP in liver (Hoehle *et al.*, 2009, Rad *et al.*, 2010) where the majority of BMP is glucuronised in F-344 rats. It is also consistent with findings of carcinogenic activity of BMP in rats in many organs including bladder but not liver (Dunnick *et al.*, 1997, National Toxicology, 1996).

In vivo studies: In the NTP-report an OECD TG 474 mouse peripheral blood micronucleus (MN) test was performed in B6C3F1-mice. Males and females were given BMP through feed (0, 25, 50, 100, 200 and 400 mg/kg bw) for 13 weeks giving rise to significant dose-reponse related increases in MN in both sexes, at the two highest dose groups of male mice (200 and 400 mg/kg bw) and the three highest dose groups of female mice (100 to 400 mg/kg bw).

A OECD TG 474 Mouse bone marrow MN test was performed in B6C3F1 mice. The study consist of two parts; Study 1 using oral gavage (100-400 mg/kg BMP) in two trials in males and Study 2 with ip injection of 150-600 mg/kg BMP in males and females. Study 1 was negative in one trial and positive in the second trial, and was concluded as inconclusive. In study 2 a significant dose-response related induction of MN was observed in females. Moreover a significant increase was also

seen in males, although it was not dose-related. Altogether the conclusion of the bone marrow MN test was considered positive.

The systematic literature search gave rise to identification of an *in vivo* comet assay (Wada *et al.*, 2014). BMP (> 98% purity) were administered orally to male Sprague-Dawley rats at two consecutive days. DNA damage (% tail DNA) in urinary bladder was significantly increased in animals treated with BMP at the high dose of 600 mg/kg/day; $p<0.05$, with no signs of toxicity. Similar to observations in in vitro comet study (Kong *et al.*, 2013), no statistical increase was seen in the liver.

No germ cell mutagenicity studies are included.

Availability to germ cells: Treinen & al (1989) reveals that BMP leads to reduced fertility, specific effect on female reproductive capacity. BMP is not a selective reproductive toxicant, because these findings are concomitant with general toxicity. .However, Bolon et al., (1997) show significantly and dose-response related reduction in follicle numbers in both F0 and F1 mice from the same experiment and there are indications of reduced follicle numbers. Moreover the reduction in follicle numbers occur also at the mid dose in F1 mice not mediating clear reproductive effects or overt body weight decrease. This indicates that BMP reaches the germ cells.

10.5.2 Comparison with the CLP criteria

Classification criteria

CATEGORY 1: "Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans."

Category 1A: "The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans."

No epidemiological studies are available so Cat 1A is not justified

Category 1B: According to CLP to classify a compound as Cat 1B the following criteria must be fulfilled: "The classification in Category 1B is based on: – positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or – **positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells.**."

Category 2: Classification criteria for category 2, from CLP: "Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: – Positive evidence obtained from experiments

in mammals and/or in some cases from in vitro experiments, obtained from: – Somatic cell mutagenicity tests in vivo, in mammals; or – Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays"

Rationale for decision on classification in Cat 1B: The in vivo tests performed are collectively positive. In the micronucleus (MN) test done in the 13-week NTP-study in B6C3F1 mice clear evidence of dose-related increases in MN in both sexes were found. These results are corroborated by the short term in vivo MN-test done in the same mouse strain given BMP by intraperitoneal injection showing that females are more susceptible than males. Moreover the *in vivo* comet assay supported the positive findings with significantly increased levels of DNA damage in bladder of SD rats. For BMP the majority of the tests performed *in vitro* give rise to positive results with three positive and one negative Ames-tests, one positive chromosome aberration test, one equivocal sister chromatid exchange test and two positive *in vitro* comet assays in urothelial cells and greatly support the positive findings in the *in vivo* studies. In summary there are positive results form *in vivo* somatic cell mutagenicity tests in mammals. Data from reproductive reports suggests that BMP reaches the gonads (Bolon et al., (1997) and Treinen et al., (1989)), supporting the classification as a male germ cell mutagen.

10.5.3 Conclusion on classification and labelling for germ cell mutagenicity

A classification as category 1B is warranted

10.6 Carcinogenicity

Table 14: Summary table of animal studies on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
2-year rodent feed study OECD TG, 453 Reliability score 1	FR-1138: BMP Purity 78.6% BMP 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane 6.9% 2,2-bis(bromomethyl)-1-	F-344 rats B6C3F1 mice Feeding with diets containing BMP F344 rats: 0, 2.500, 5.000, or 10.000 ppm in feed equivalent to: Males: 0, 100, 200 and 430 mg/kg bw Females: 0, 115, 230, 460 mg/kg bw A stop-exposure group	**F-344 rats**: Clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas) and at 4 sites in female rats (mammary gland, oral cavity, esophagus and thyroid gland). Dose response relationships between exposure and carcinogenicity were	(Dunnick *et al.*, 1997) (National Toxicology Program, 1996)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	bromo-3-hydroxypropane 0.2% pentaerythritol 7.7% dimers and structural isomers	receiving 20000 ppm (800 mg/kg bw) BMP for three months and then continued on normal diet B6C3F1 mice: 0, 312, 625, or 1.250 ppm in feed equivalent to: Males: 0, 35, 70, 140 mg/kg bw Females: 0, 40, 80, 170 mg/kg bw	evident for several tumour types. Most cancer-sites are relevant for humans. Survival of 5.000 and 10.000 ppm males and females and 20.000 ppm stop-exposure males was significantly lower than controls. Mean body weights of rats receiving 10.000 ppm and stop-exposure males receiving 20.000 ppm were lower than controls (5-15%). Food consumption was generally similar to that by controls, except from stop-exposure males. **B6C3F1 mice**: Clear exposure related carcinogenic effects at 3 sites in the male (lung, kidney and Harderian gland) and female mice (subcutaneous tissue, lung and Harderian gland). Dose response relationships between exposure and carcinogenicity was evident for several tumour types. Most sites of cancer are relevant for humans. Survival of 1.250 ppm males and females was significantly lower than that of the controls. Mean body weights of exposed male and female mice were similar to controls throughout the study. Final mean body weights were also generally similar to those of controls. Feed consumption by exposed male and female mice was similar to that by controls.	
2-year rodent feed study OECD TG 453 Reliability score 2	FR-1138 containing 80% BMP 8% tribromeopentyl alcohol 6% monobromopentyl tricol	Sprague-Dawley (SD) rats Male and females were treated with 0, 5, 100 mg FR-1138/kg/day	The incidence rates of all tumor types in any of the dose groups were comparable to the control group. Toxicity: Rats given high dose had some evidence of toxicity, including organ weight changes (liver in males; kidneys in females) and degenerative changes in the liver, eyes and possibly thyroid gland Mortality: No treatment-related changes	(Keyes *et al.*, 1980)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	3% other impurities		Body weights: No treatment related changes	

Table 15: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Feed study OECD TG 453 2 year study	FR-1138: BMP Based on the same NTP study as reported by Elwell et al., 1989	B6C3F1 mice: 0, 312, 625, or 1.250 ppm in feed equivalent to: Males: 0, 35, 70, 140 mg/kg bw Females: 0, 40, 80, 170 mg/kg bw DNA was isolated from lung neoplasms and analysed to obtain information regarding the frequency and spectrum of K-ras mutations.	Exposure of B6C3F1 mice to BMP led to the induction of significantly higher frequency of lung neoplasms which correlated with the incidence of K-ras mutations in the corresponding tumors as compared to the feed control mice. The development of lung tumors may involve multiple carcinogenic processes including direct DNA damage and/or indirect DNA damage.	(Ton *et al.*, 2004) (National Toxicology Program, 1996)
Feed study or oral gavage, 90-day study 13 week studies	Purity: Not specified	F-344/N rats B6C3F1 mice GAVAGE: Rats were dosed with daily doses of 0, 50, 100, 200, 400 and 800 mg/kg. Mice were dosed with daily doses of 0, 25, 50, 100, 200, and 400 mg/kg. FEED: BMP was mixed in feed at 0, 1.250, 2.500, 5.000, 10.000, and 20.000 ppm for rats equivalent to 0, 68, 135, 300, 640 and 1.440 mg/kg bw in males and 0, 80, 148, 310, 630 and 1.340 mg/kg bw in females and at 0, 625, 1.250, 2.500, 5.000 and 10.000 ppm for mice equivalent to 0, 113, 235, 690, 1.750 and 5.850 mg/kg bw in males and 0, 174, 473, 988, 2.155 and	Transitional cell hyperplasia of the urinary bladder was seen in male rats exposed by oral gavage (400 and 800 mg/kg bw) or in feed (1.340 mg/kg bw) and in both sexes of mice exposed through feed (males: 690, 1.750 and 5.850 mg/kg bw; females: 2.155 and 4.190 mg/kg bw). Mice are more sensitive than rats, and males are more sensitive than females.	(Elwell *et al.*, 1989) (National Toxicology Program, 1996)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		4.190 mg/kg bw in females Necropsy was performed within one day of the last dose.		

Table 16: (from the NTP report)

Summary of Site-Specific Carcinogenic Effects in Rats and Mice in the 2-Year Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol

	Male Rats	Female Rats	Male Mice	Female Mice
Site				
Skin	+	−	−	−
Subcutaneous tissue	+	−	−	+
Mammary gland	+	+	−	±
Zymbal's gland	+	−	−	−
Oral cavity	+	+	−	−
Esophagus	+	+	−	−
Forestomach	+	−	±	±
Small intestine	+	−	−	−
Large intestine	+	−	−	−
Mesothelium	+	−	−	−
Kidney	±	−	+	−
Urinary bladder	+	−	−	−
Lung	+	−	+	+
Thyroid gland	+	+	−	−
Seminal vesicle	+	NA	−	NA
Hematopoietic system	+	−	−	−
Pancreas	±	−	−	−
Harderian gland	−	−	+	+
Circulatory system	−	−	−	±

\+ = some or clear evidence
± = equivocal evidence
− = no evidence
NA = not applicable

Table 17: (from the NTP report)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 2,2-Bis(bromomethyl)-1,3-propanediol

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 2,500, 5,000, or 10,000 ppm and 20,000 ppm stop-exposure (equivalent to 0, 100, 200, or 430 mg/kg and 800 mg/kg)	0, 2,500, 5,000, or 10,000 ppm (equivalent to 0, 115, 230, or 460 mg/kg)	0, 312, 625, or 1,250 ppm (equivalent to 0, 35, 70, or 140 mg/kg)	0, 312, 625, or 1,250 ppm (equivalent to 0, 40, 80, or 170 mg/kg)
Body weights	10,000 ppm and 20,000 ppm stop-exposure groups lower than controls	10,000 ppm group lower than controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	26/51, 20/53, 13/51, 1/55, 0/60	36/50, 27/51, 23/53, 5/52	42/50, 36/51, 35/50, 30/48	37/52, 30/50, 26/51, 11/50
Nonneoplastic effects	Kidney: focal atrophy (0/51, 0/53, 0/51, 5/55, 0/59); papillary degeneration (0/51, 5/53, 30/51, 29/55, 16/59); papillary epithelial hyperplasia (10/51, 20/53, 25/51, 47/55, 21/59); pelvis, transitional epithelium, hyperplasia (0/51, 0/53, 0/51, 4/55, 4/59) Lung: alveolar/ bronchiolar hyperplasia (3/51, 4/53, 5/51, 7/55, 14/60) Thyroid gland: follicular cell hyperplasia (1/51, 0/53, 2/51, 5/55, 6/59) Seminal vesicle: hyperplasia (1/51, 6/53, 4/51, 16/55, 33/60) Pancreas: focal hyperplasia (3/51, 9/53, 12/51, 14/53, 27/59) Forestomach: mucosal hyperplasia (4/51, 12/53, 6/51, 6/55, 6/59) Urinary bladder: hyperplasia (0/51, 0,53, 1/51, 3/55, 10/59)	Kidney: focal atrophy (0/50, 2/51, 1/53, 7/52); papillary degeneration (0/50, 1/51, 3/53, 17/52); papillary epithelial hyperplasia (0/50, 1/51, 1/53, 7/52)	None	Lung: alveolar epithelium, hyperplasia (1/52, 3/50, 8/51, 15/50)

Table 17 continued: (from the NTP report)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects (continued)	Lung: alveolar/ bronchiolar adenoma or carcinoma (1/51, 1/53, 3/51, 4/55, 7/60); squamous cell carcinoma (0/51, 0/53, 0/51, 0/55, 3/60) Thyroid gland: follicular cell adenoma or carcinoma (0/51, 2/53, 6/51, 3/55, 9/59) Seminal vesicle: adenoma or carcinoma (0/51, 0/53, 0/51, 0/55, 2/60) Hematopoietic system: mononuclear cell leukemia (27/51, 29/53, 40/51, 34/55, 25/60)			
Uncertain effects	Kidney (renal tubule): adenoma (0/51, 0/53, 1/51, 3/55, 1/59) Pancreas: acinar cell adenoma (1/51, 2/53, 4/51, 3/53, 3/59)	None	Forestomach: squamous cell papilloma or carcinoma (0/50, 3/51, 3/50, 4/49)	Mammary gland: carcinoma (0/52, 0/50, 1/51, 3/50) Forestomach: squamous cell papilloma (0/52, 1/50, 5/51, 3/50) Circulatory system: hemangioma and hemangiosarcoma (1/52, 2/50, 0/51, 5/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

10.6.1 Short summary and overall relevance of the provided information on carcinogenicity

Table 18: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Fischer 344 rat (Dunnick et al., 1997) (National Toxicology Program,	Several tumor types relevant for humans	Males: + Females: +	Males: + Females: +		Both	No	Oral, diet	Yes

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
1996)								
B6C3F1 mice (Dunnick et al., 1997) (National Toxicology Program, 1996)	Several tumor types relevant for humans	Males: + Females: +	Males: + Females: +		Both	No	Oral, diet	Yes
SD rat Keyes et al., 1980	-	-	-	-	-	No	Oral, diet	-

With respect to ADME, clear differences exist between rodents and humans. The rate of BMP glucuronidation, the predominant metabolic conversion, in rodent cells was 150-fold higher than in human hepatocytes. Although the ADME-studies clearly describe the fate of BMP in rat the mechanism(s) for the carcinogenic potential of BMP is not established.

The test material in the NTP study, FR-1138, is reported to contain the following major impurities: 6,6% monobromoneopentyl triol (CAS no 19184-65-7), 6,9% tribromoneopentyl alcohol (CAS no 36483-57-5/1522-92-5; hereafter referred to as CAS no 36483-57-5 , and other minor impurities besides 78,6% BMP.
The monobromoneopentyl triol (CAS no 19184-65-7) is listed in the ECHA Annex III inventory (Annex III: Criteria for substances registered in quantities between 1 and 10 tonnes. ECHA compiled an inventory of substances likely to meet the criteria of Annex III to the REACH Regulation. The aim is to support registrants in identifying whether reduced minimum information requirements or a full Annex VII information set is required. The inventory shows indications for concern) as a suspected mutagen and a suspected carcinogen, mostly with moderate reliability. No more data is readily available for this compound.

The report 'Category approach for selected brominated flame retardants' (Danish EPA, 2016; (Wedebye *et al.*, 2016)) states that according to (Q)SAR-based clusterings BMP and 36483-57-5 (the 6,9% impurity) are in the same (Q)SAR-based clusters for carcinogenicity and genotoxicity. Both have been tested in the USEPAs NTP program. There are genotoxicity study results revealing almost identical properties for both BMP and 36483-57-5. The substance with CAS no. 36483-57-5 showed no evidence of mutagenic activity in the absence or presence of rat liver S9, but showed a clear evidence of mutagenic activity in strains TA100 and TA1535 in the presence of hamster liver S9. It also gave positive results in the mouse lymphoma assay

in the presence of rat liver S9. Increases in chromosomal aberrations were induced in cultured peripheral human lymphocytes in the presence of metabolic activation, and at the highest test substance concentration in the absence of metabolic activation. The substance did not induce any marked or significant increases in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat liver cells following in vivo exposure and therefore, the substance was considered to be non-genotoxic in this study. Furthermore, the substance did not increase the frequency of micronucleated polychromatic erythrocytes in the bone marrow in mice.

In the 2-year NTP-study reported by Dunnick et al., 1997, F-344 rats and B6C3F1 mice were given BMP orally through feed (Dunnick *et al.*, 1997, National Toxicology Program, 1996). The main results are summarised in Table 16. Male rats were given 100, 200 or 430 mg BMP/kg/day whereas female rats were given 115, 230 or 460 mg BMP/kg/day, plus a stop-exposure group at 800 mg BMP/kg/day (3 months exposure). Clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas) and at 4 sites in female rats (mammary gland, oral cavity, esophagus and thyroid gland) (Table 17). Dose response relationships between exposure and carcinogenicity were evident for several tumour types, and most cancer-sites are relevant for humans. Survival at the two highest doses in males and females and the male stop-exposure group was significantly lower than controls. Mean body weights of rats receiving the highest dose and the stop-exposure group in males were lower than controls (5-15%). Food consumption was generally similar to that by controls, except from stop-exposure males.
Male mice were given 35, 70, or 140 mg BMP/kg/day whereas female mice were given 40, 80 or 170 mg BMP/kg/day.). A clear exposure related carcinogenic effects at 3 sites in the male (lung, kidney and Harderian gland) and female mice (subcutaneous tissue, lung and Harderian gland) (Table 17). Dose response relationships between exposure and carcinogenicity was evident for several tumour types and most sites of cancer are relevant for humans. Survival of the high dose males and females was significantly lower than that of the controls. Mean body weights of exposed male and female mice were similar to controls throughout the study. Final mean body weights were also generally similar to those of controls. Feed consumption by exposed male and female mice was similar to that by controls.

In the 90-day study by Elwell et al., 1989 (13-weeks study of the NTP-study) it was shown that kidney and bladder are target organs, that mice are more sensitive than rats for the development of kidney and bladder lesions, and that males are more sensitive than females for the development of renal papillary degeneration or necrosis. In this study, hyperplasia was induced at the high doses, with potential to be transformed into tumours. This is supportive evidence for the carcinogenic effect of BMP seen in Dunnick et al., 1997.

Keyes et al., 1980, reported no carcinogenic effects in male or female Spraque-Dawley rats when BMP was administered in the feed at doses delivering 5 or 100 mg/kg/day. In the study by Dunnick et al., F344 rat were given 2.500, 5.000 or 10.000 ppm delivering approximately 100, 200 or 400 mg BMP/kg/day. Thus the low dose in Dunnick et al was approximately equivalent to the highest dose in the Keyes study. In the Dunnick et al treatment-related neoplasms occurred at the low dose (100 mg/kg) in subcutaneous tissues and oral cavity in male F-344 rats and in the mammary gland of male and female F-344 rats. As the dose increased, a wider spectrum of carcinogenic responses occurred in the rats. The typically higher background incidences of mammary tumors in Sprague-Dawley rats, or perhaps metabolic differences, may have accounted for the absence of a detectable carcinogenic effect in this rat strain.

The validity of lung cancers observed in the B6C3F1 mice of the NTP-study was confirmed by mutation characterization in the target gene K-ras (Ton et al., 2004). The induced mutations *may* indicate genotoxicity of parent compound or metabolites*, directly or indirectly*. A relative high increase in K-ras codon 12 G → T transversions (CGT to GTT) was observed in BMP-induced lung neoplasms compared to historical control.

In summary, BMP was tested for carcinogenicity as a commercial mixture (FR-1138R) containing ~80% of the parent compound in one experiment in mice and in two experiments in rats by oral administration in the diet. In mice, it increased the incidence of tumours of the Harderian gland, forestomach and lung in both males and females and of subcutaneous sarcomas in females. In the NTP study in male rats, it increased the incidences of tumours of the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, oesophagus, forestomach, small and large intestine, peritoneum, lung and thyroid. In female rats the incidences of oesophageal, mammary gland and thyroid follicular tumours were increased. In another study with SD rats and lower doses of BMP with higher purity, no carcinogenic effect was observed. Several tumor types are relevant for humans and they appear at doses not inducing excessive general toxicity. Histopathological changes were observed in the kidney and the urinary bladder of rats and mice administered BMP for 90 days. QSAR data show that clustered compounds with similar reactive molecular groups have mutagenic and carcinogenic properties, in line with the experimental data. In vitro, the rate of glucuronidation by rat hepatic microsomes was 90-fold greater than that of human hepatic microsomes. Moreover, there is evidence of mutagenic activity *in vivo*.

10.6.2 Comparison with the CLP criteria

Classification category 1: Known or presumed human carcinogens.

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Such evidence may be derived from: – human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or – animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

CATEGORY 2: Suspected human carcinogens. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

There is sufficient evidence with the reliable NTP-study in both rats and mice, giving chemical- and dose-related inductions of multisite human relevant tumors, in both sexes, which warrants classification of BMP as Carc 1B.

10.6.3 Conclusion on classification and labelling for carcinogenicity

Carc 1A: no epidemiological studies available, not justified

Carc 1B: Based on clear evidence in animals. Justified for BMP

Carc 2: Based on some evidence in animals.

10.7 Specific target organ toxicity-single exposure

Not evaluated in this dossier

10.8 Specific target organ toxicity-repeated exposure

Not evaluated in this dossier

10.9 Aspiration hazard

Not evaluated in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier

13 ADDITIONAL LABELLING

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15 ANNEXES

Annex I to the CLH report