Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

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

EC Number: 221-967-7

CAS Number: 3296-90-0

CLH-O-0000001412-86-212/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2018

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: -

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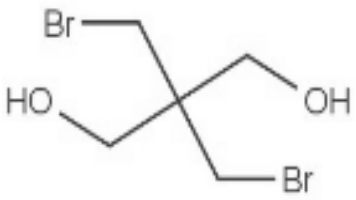
Version number: 1.2 **Date: 07.04.2017**

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
-				

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

RAC general comment
The Dossier Submitter (DS) proposed the classification of 2,2-bis(bromomethyl)propane-1,3-diol (BMP), a flame retardant used in plastics and as an intermediate for production of other chemicals, for mutagenicity (Cat. 1B) and carcinogenicity (Cat. 1B).

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	-		

Property	Value	Reference	Comment (e.g. measured or estimated)
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 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]	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.	(Hoehle *et al.*, 2009) ECHA dossier: https://echa.europa.eu/registration-dossier/-/registered-dossier/7873/7/2/2

Method	Results	Remarks	Reference
	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 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.	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. 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.	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	(Rad *et al.*, 2010) This is a follow-up study of the study above.

Method	Results	Remarks	Reference
	[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 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).	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) 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	

Method	Results	Remarks	Reference
		glucuronidation activity were calculated, and the data for the kinetic studies were subjected to analysis based on Michaelis-Menton kinetics.	

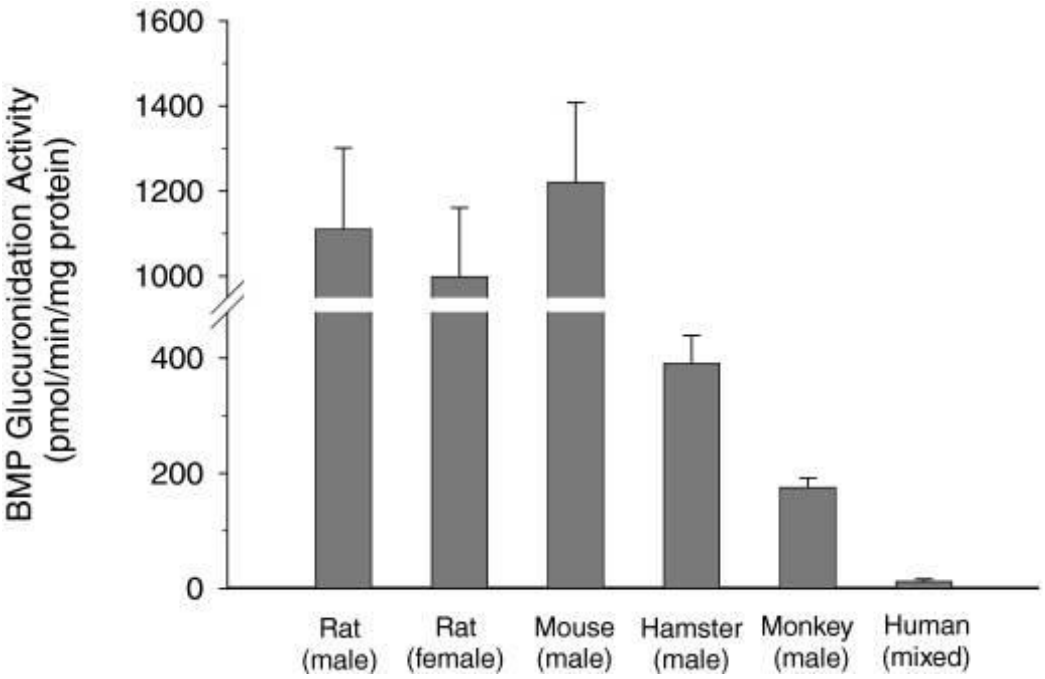

Figure 1 *Figure 6 from Rad et al., 2010. Activities of hepatic microsomes from male and female F-344 rats, male B6C3F1 mice, male Golden Syrian hamsters, male rhesus monkeys, and humans (mixed gender) for the glucuronidation of BMP. Rates of glucuronidation were determined at 50 μM BMP and are expressed 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	FR522 (2,2-Bis(bromomethyl)-1,3-propanediol	Initial test: Strain TA100 +/- S9-mix from rat and hamster over a wide dose range (0-5000 ug/plate).	**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**
Mutation Assay GLP Reliability score 2	Purity: 99.5 (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	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	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)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Bacterial Reverse Mutation Assay Reliability score 1	Purity: 96,3% 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	Main test: Strains TA98, TA100, TA1535 and TA1537 Metabolic activation: Aroclor 1254-induced S9-mix from Sprague-Dawley rat and male Syrian hamster livers in 10% concentrations.	**Conclusion: Negative** 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	(Mortelmans *et 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.	

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vitro Sister Chromatid Exchange Assay in Mammalian Cells Reliability score 1	Purity: Not 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	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 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).	**Conclusion:** **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	**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**
		Technologies, Rockville, 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)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vivo mammalian alkaline comet assay OECD TG 489 Reliability score 1	Purity: >98.0% Dosage: 0, 300 or 600 mg/kg bw	Animal strain: Male 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.	**Conclusion:** **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.	(Wada *et al.*, 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	BMP Dow Chemical Company (Midland, MI), LotNo. MM	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.	(Treinen *et al.*, 1989)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
protocol, part of the NTP 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	05137-636, purity 87.3%. 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.	At the highest dose, BMP caused a body weight decrease in the F0 animals of both sexes with no effect 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).	National Toxicology Program, 1996
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

RAC evaluation of germ cell mutagenicity

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS came to the conclusion that a proposal to classify BMP as Muta. 1B is justified on the basis of the available *in vitro/in vivo* genotoxicity data and indications that BMP reaches the germ cells.

In vitro tests

The majority of the available *in vitro* genotoxicity tests conducted with BMP were positive, including three bacterial gene mutation tests (Unknown author, 1996a,b; Zeiger *et al.*, 1992), one chromosomal aberration test (Galloway *et al.*, 1987) and two comet assays (Kong *et al.*, 2011; Kong *et al.*, 2013).

One bacterial gene mutation test was negative (Mortelsmans *et al.*, 1986) and one sister chromatid exchange assay resulted in an equivocal outcome (Galloway *et al.*, 1987).

In vivo tests (somatic cells)

A micronucleus test in peripheral blood cells was positive in male and female mice after feeding with BMP (NTP, 1996). Further micronucleus tests in bone marrow cells of mice showed an inconclusive result in males after oral gavage of BMP, whereas a positive result was observed in males and females after intraperitoneal (i.p.) injection of BMP (NTP, 1996).

An *in vivo* comet assay was positive in urinary bladder cells and negative in liver cells of rats given BMP by gavage (Wada *et al.*, 2014).

In vivo tests (germ cells)

No validated germ cell mutagenicity tests are available.

Treinen *et al.* (1989) showed that BMP exposure leads to reduced fertility, as a specific effect on female reproductive capacity of mice, whereas Bolton *et al.* (1997) observed a reduction in follicle numbers of female mice. The DS concluded that these findings indicate that BMP reaches the germ cells.

Conclusion

On the basis of an analysis and assessment of the available genotoxicity studies with BMP the DS concluded that BMP induces mutagenic effects *in vitro* (in bacteria and in mammalian cell cultures) as well as *in vivo* (in somatic cells of mammals). Additional data from reproductive reports led to the conclusion that BMP reaches the germ cells. Due to the *in vivo* mutagenicity in somatic cells and the information on systemic availability of BMP, there was some evidence that the substance also has potential to cause mutations in germ cells. Therefore, the DS considered that classification as Muta. 1B (H350) is warranted.

Comments received during public consultation

Two MSCAs agreed with the proposal for classification of BMP as Muta. 1B, while another two MSCAs were of the opinion that classification as Muta. 2 would more be appropriate. For one MSCA, mutagenicity in somatic cells was adequately shown but they considered that there were doubts concerning whether classification as Muta. 1B is appropriate.

Assessment and comparison with the classification criteria

In vitro/in vivo tests with somatic cells

The evaluation of the available genotoxicity data on BMP by the DS and RAC resulted in the same conclusion.

Based on consideration of reliable genotoxicity data, RAC also concludes that the substance induces mutagenic effects *in vitro* as well as *in vivo* in somatic cells.

In vivo tests with germ cells

No germ cell mutagenicity studies with BMP are available.

Other data indicating that the germ cells may be reached

Toxicokinetic data

The only available toxicokinetic data on BMP concentrations in the gonads was given in the publication of Hoehle *et al.* (2009). In the testis of rats only 0.01% BMP was recovered after up to 10 days of exposure. No female rats were used in study.

There were no other toxicokinetic data examining whether BMP reaches the ovaries of mammals.

No data were available on the distribution of the metabolite(s) before and after interal reabsorption, or whether BMP or its glucuronide metabolite is the active compound.

Indications from other studies

Reproductive studies in mice by Treinen *et al.* (1989) and Bolon *et al.* (1997) identified the ovary as a target organ following BMP exposure.

Bolon *et al.* (1997) characterised altered follicle counts as quantifiable endpoints of ovarian injury. BMP, one of the substances studied, significantly decreased the differential counts of follicles in CD-1 mice. The reductions in follicle numbers were proportional to tested doses.

Treinen *et al.* (1989) demonstrated that BMP impaired fertility in female CD-1 mice in a continuous breeding study (in which treatment started 7 days prior to and during a 98 cohabitation period, and the neonates were removed to encourage the next pregnancy (Task 2)), in crossover mating experiments (Task 3) and in the second generation (Task 4).

The significantly reduced numbers of litters per pair during the whole treatment period of the continuous breeding experiment (Task 2) could indicate increased cycle lengths (but there were no data on cycle duration for this group). The authors stated that effects on oestrous cyclicity were not seen in treated females in the crossover experiment (Task 3) and in the second

generation (Task 4). The absence of consistent effects was interpreted that no clear conclusion on the effect on oestrous cyclicity can be drawn.

BMP exposure significantly decreased the numbers of litters per pair, pups born alive per litter, and pup weight when adjusted for litter size in this study. Sperm concentration, motility and morphology in male CD-1 mice were unaffected by BMP exposure. These results suggested the conclusion that BMP (or its active metabolite(s)) could reach the ovaries of female CD-1 mice. It may not have reached (or had only reached in insufficient amounts) or affected the testes of male CD-1 mice.

The number of live pups per litter were significantly lower in the continuous breeding experiment (Task 2), the crossover experiment (Task 3) and the second generation experiment (Task 4) in the Treinen study (Treinen *et al.*, 1989). The lower number of live pups may be interpreted as being consistent with the reduced follicle counts published in the study of Bolon *et al.* (1997), which is assumed to have documented the results of the same test animals (CD-1 mice) from the same NTP laboratory. The dose-related decrease in differential follicle counts in treated mice, the markedly reduced follicle numbers at the high dose (0.4%, i.e. 589 mg/kg bw/d) and the consistency between the results of the two studies gives a strong indication that this is a treatment-related effect.

However, the lower body weight gains of dams in comparison to the control values seen in Tasks 2, 3, and 4 may raise the question whether the effects on the follicles could be secondary to lower body weight. At the highest dose (0.4%, i.e. 589 mg/kg bw/d) the final body weights after continuous breeding (Task 2) were 11-15% lower than the control values, 17% lower in the crossover mating group (Task 3), and 18% lower in the second generation (Task 4). As the numbers of live pups per litters were reduced in all experiments, the reduced body weight gain is likely to have contributed to the smaller litters. Some uncertainty remains as no data on corrected body weights in dams were provided.

One MSCA in their comment suggested that the decrease in follicle counts could be secondary to other effects such as a disruption of the hypothalamic-pituitary axis. The available data from the combined chronic toxicity/carcinogenicity studies in rats and mice do not support this assumption. Although such effects can not be excluded, no indication of effects on the hypothalamic-pituitary axis were observed from the 13 week studies in rats and mice or from the organs at sites where tumours were seen (NTP, 1996).

Based on these data, two conclusions can be drawn:

1. Although it cannot be ruled out with certainty that BMP has the potential to induce mutagenic effects in germ cells, there is no direct evidence of this due to the lack of specific studies on germ cell mutagenicity. There is also no direct evidence that the observed reproductive effects were due to mutations in germ cells. If no other data were available, classification as Muta. 2 would be appropriate (CLP Guidance, 3.5.2.2).
2. The DS, however, in their rationale referred to the following relevant criterion for classification as Muta. 1B in the CLP Regulation (Annex I, Table 3.5.1): "… *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*". Data from Bolon *et al.* (1997) and Treinen *et al.* (1989) indicated that BMP reaches the germ cells in female mice.

RAC considered the following arguments:

1. RAC discussed the proposal under the premise given in the CLP Guidance (3.5.2.4): *"It could be argued that in a case where in vivo mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, than that substance should also be considered as a Category 1B mutagen*". According to RAC's interpretation, the criteria define that evidence for reaching the germ cells in combination with evidence for *in-vivo* mutagenicity may be sufficient to conclude that a substance is a germ cell mutagen.
2. RAC considered the arguments as to whether the data on systemic availability and whether the germ cells were reached were sufficiently robust. It was acknowledged that evidence mainly comes from two sources: (a) the observation of tumours at multiple sites, which was reflected in RAC's proposal to classify BMP as carcinogen Cat. 1B and (b) the effects on the ovarian cells which, although not sufficient for showing that in germ cells mutagenic effects as such have occurred, but demonstrated that the germ cells were reached. The evidence that toxicokinetic studies found a low percentage of the radioactively labelled substance in the testis was considered as supporting evidence that the germ cells were reached. This finding, however, in this case was of lower importance. Unfortunately, no toxicokinetic data were available for female animals.
3. It was noted that BMP impaired fertility, as indicated from the Treinen *et al.* study (1989). A separate documentation of all the data on

reproductive toxicity would have been useful, however in the DS proposal a classification proposal only considered selected endpoints. Dams showed lower body weight at the time of parturition (Task 3 and Task 4), for which the lower numbers of live pups/litter cannot be excluded as a cause (as no corrected body weight data were available). Body weight effects linked to lower pup numbers made maternal toxicity appear less likely as being responsible for the effect on follicles. Based on the available data, there is no indication of a hormone-related effect.

4. It might appear inconsistent that no ovary effects were observed in other repeated dose toxicity studies (e.g. from the NTP report). However, these studies did not include morphometric analyses of small and growing follicles and the absence or presence of effects on follicle development remains uncertain. Finally, the reduced numbers of small and growing follicles were reproduced in two sets of studies in the Bolon *et al.* publication (1997) and showed dose-dependency. No arguments were found to disregard the evidence (from the cancer studies and from studies of Treinen/Bolon) that female germ cells were reached and that the substance interacted with these cells.

In conclusion, RAC agreed with the DS's proposal that there is positive evidence of somatic cell mutagenicity from *in vitro/in vivo* studies and evidence from the reproductive toxicity studies support that BMP reaches the (female) germ cells. Both facts in combination are sufficient to give 'some' evidence that the substance has the potential to cause mutations to germ cells.

Therefore, RAC agrees with the DS' conclusion that BMP should be classified as a **germ cell mutagen, Cat. 1B; H340**.

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(hydroxy methyl)-1-bromo-3-	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:	**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	(Dunnick *et al.*, 1997) (National Toxicology Program, 1996)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	hydroxypropane 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane 0.2% pentaerythritol 7.7% dimers and structural isomers	Males: 0, 100, 200 and 430 mg/kg bw Females: 0, 115, 230, 460 mg/kg bw A stop-exposure group 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	female rats (mammary gland, oral cavity, esophagus and thyroid gland). Dose response relationships between exposure and carcinogenicity were 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	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	(Keyes *et al.*, 1980)

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	6% monobromoopentyl tricol 3% other impurities		changes in the liver, eyes and possibly thyroid gland Mortality: No treatment-related changes 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	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
		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 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	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
Program, 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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS summarised two carcinogenicity studies; one study in rats and mice performed by the National Toxicology Program (NTP) and one study in rats performed by industry. Two supportive studies were also included, one mechanistic follow-up study to the NTP-study and one 90-day repeated dose toxicity study in rats and mice.

2-year rat/mouse study (Dunnick et al., 1997; NTP, 1996)

In the carcinogenicity study by the NTP, conducted under GLP and in conformity with OECD TG 453, F-344 rats and B6C3F1 mice were dosed with the technical mixture FR-1138 orally through feed. The FR-1138 contained 78.6% 2,2-bis(bromomethyl)propane-1,3-diol (BMP); 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol and 7.7% structural isomers and dimers.

Rats

F-344 rats (60/sex/dose) were provided FR-1138 in the diet at 0, 2500, 5000 or 10000 ppm (males: 0, 100, 200, 430 mg/kg bw/day; females: 0, 115, 230, 460 mg/kg bw/day) for 2 years. A stop-exposure group was also included where 60 male rats received 20000 ppm FR-

1138 (800 mg/kg bw/day) for 3 months and then fed control diet for the remainder of the study.

General toxicity consisted of decreased body weight gain and terminal body weight in the exposed groups as compared to the controls, resulting in mean terminal body weights up to 14% lower in males in the two high dose groups and up to 5% lower in females in the high dose group. Food consumption was generally similar to that of controls. Decreased survival occurred in the mid and high dose males and females primarily due to treatment-related neoplasms. Clinical findings consisted of cutaneous and subcutaneous tissue masses on the face, tail and on the dorsal and ventral surfaces of exposed rats.

The DS reported treatment-related neoplastic and non-neoplastic lesions combined for 17 tissues in male rats (skin, subcutaneous tissue, Zymbal gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, peritoneum, kidney, urinary bladder, lung, thyroid, seminal vesicle, haematopoietic system, mammary gland and pancreas) and five tissues in female rats (mammary gland, oral cavity, oesophagus, kidneys and thyroid). The data indicated dose-response relationships for several of these tumours. The effects observed in the kidney and pancreas of male rats were predominantly hyperplastic, thus the DS considered the effects in these tissues of male and female rats as equivocal with regard to carcinogenicity and reported *clear or some evidence* of treatment-related carcinogenic effects in 15 tissues in male rats and four tissues in female rats (Table below).

Mice

B6C3F1 mice (60/sex/dose) were given FR-1138 in the diet at 0, 312, 625, 1250 ppm (males 0, 35, 70, 140 mg/kg bw/day; females 0, 40, 80, 170 mg/kg bw/day) for 2 years.

Decreased survival was reported at the highest dose in males and females, primarily due to treatment-related neoplasms. Mean body weights of exposed male and female mice as well as food consumption were similar to controls throughout the study. Clinical findings in exposed mice consisted of swelling, discharges and tissue masses in the eyes (related to the tumours of the Harderian gland).

The DS reported treatment-related neoplastic and non-neoplastic lesions combined for four tissues in males (Harderian gland, lung, forestomach and kidney) and 6 tissues in females (Harderian gland, lung, subcutaneous tissue, forestomach, mammary gland and circulatory system) and the data indicated a dose-response relationships for several tumour types. The DS reported *clear or some evidence* of treatment-related carcinogenic effects in four of the tissues in male mice and three of the tissues in female mice (Table below).

Table: Summary table of carcinogenic effects in 2-year studies of BMP in rats and mice (Dunnick et al., 1997; NTP, 1996).

Site	**Rats**		**Mice**	
	Male	**Female**	**Male**	**Female**
Skin	+			
Subcutaneous tissue	+			+
Mammary gland	+	+		+/-
Zymbal's gland	+			
Oral cavity	+	+		
Oesophagus	+	+		
Forestomach	+		+	+/-
Small intestine	+			
Large intestine	+			

Mesothelium	+			
Kidney	+/-		+	
Urinary bladder	+			
Lung	+		+	+
Thyroid gland	+	+		
Seminal vesicle	+			
Haematopoietic system	+			
Pancreas	+/-			
Harderian gland			+	+
Circulatory system				+/-

+ = some or clear evidence of carcinogenic activity; +/- = equivocal evidence of carcinogenic activity

The DS considered the outcome of this NTP-study as evidence of carcinogenicity of BMP in rodents. In the technical mixture FR-1138, containing ~80% BMP, the two main impurities were monobromoneopentyl triol (6.6%) and tribromoneopentyl triol (6.9%). The DS referred to ECHA screening, for substances likely to fulfil the criteria for inclusion in REACH Annex III, which showed indications for concern for monobromoneopentyl triol as a suspected mutagen and carcinogen. Studies for BMP and tribromoneopentyl triol from the NTP-program showed almost identical genotoxicity properties. Altogether, the DS considers these major impurities to have similar properties to BMP.

2-year rat study (Keyes et al., 1980)

Sprague-Dawley rats (49-50/sex/dose) were given FR-1138 in the diet at 0, 5 or 100 mg/kg bw/day for 2 years. The FR-1138 was reported to contain 80% BMP, 8% tribromeopentyl alcohol, 6% monobromeopentyl triol and 3% of other impurities.

No evidence of carcinogenicity was reported in the study. General toxicity was low, with only slight reductions in body weight in treated males. Survival was not significantly different among the groups. The high dose, 100 mg/kg bw/day, is comparable to the low-dose in the NTP-study described above (Dunnick *et al.*, 1997; NTP, 1996).

Mechanistic follow-up study to the NTP carcinogenicity-study (NTP, 1996; Ton et al., 2004)

In a follow-up study to the NTP-carcinogenicity study (Dunnick *et al.*, 1997; NTP, 1996), lungs from the B6C3F1 male and female mice were fixed, DNA isolated and characterised for mutations in the protooncogene K-ras. The result showed that the frequency of mutated K-ras in BMP-induced lung neoplasms (57%) was significantly elevated over that of spontaneous lung neoplasms from control mice (15%). The DS considered this study to support the observed lung tumours and that this may indicate direct or indirect genotoxicity of BMP or its metabolites.

90-day study in rats/mice (Elwell et al., 1989; NTP, 1996)

In a 90-day dose-range finding study to the 2-year carcinogenicity study, F-344/N rats were dosed with FR-1138 either via gavage at 0, 50, 100, 200, 400 and 800 mg/kg bw/day or via the diet at 0, 1250, 2500, 5000, 10000 and 20000 ppm (males: 0, 100, 200, 400, 800, 1700 mg/kg bw/day; females: 0, 100, 200, 400, 800, 1600 mg/kg bw/day). Similarly, B6C3F1 mice were exposed to BMP at 0, 25, 50, 100, 200 and 400 mg/kg bw/day or via the diet at 0, 625,

1250, 2500, 5000 and 10000 ppm (males: 0, 100, 200, 500, 1300, 3000 mg/kg bw/day; females: 0, 140, 300, 600, 1200, 2900 mg/kg bw/day).

The results showed that the kidneys and bladder were target organs and that mice were more sensitive than rats for kidney and bladder lesions. Transitional cell hyperplasia was observed at the higher doses, with potential to be transformed into tumours. The DS considered this study as supportive evidence for the carcinogenic effect of BMP seen in the NTP carcinogenicity study.

Overall, the DS considered that there is sufficient evidence from the NTP-study of FR-1138 (containing ~80% BMP) in two species, rats and mice, showing substance and dose-related inductions of multisite human relevant tumours in both sexes, which warrants classification of BMP as Carc. 1B.

Comments received during public consultation

Five MSCA's commented on the classification proposal. All supported classification of BMP as Carc. 1B. Two MSCA's considered the preneoplastic lesions observed in two studies on rats and mice as supporting evidence. One MSCA did not consider the negative results in the industry study to weaken the strength of evidence for carcinogenicity considering the lower doses used in that study in comparison with the NTP-study.

One MSCA disagreed with the DS's statement "clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas)". The DS referred to two of these tissues, which in their view did not indicate dose-related effects, and also suggested inclusion of historical control data (HCD). The DS responded that they have cited the study authors' conclusions regarding "exposure-related carcinogenic effects" and that the HCD is available in the NTP report (NTP, 1996).

Assessment and comparison with the classification criteria

Two carcinogenicity studies were provided on FR-1138, a technical mixture containing ~80% BMP; one study in rats and mice conducted by the National Toxicology Program under GLP and in accordance with OECD TG 453 (Dunnick *et al.*, 1997; NTP, 1996) and one study in rats conducted by industry (Keyes *et al.*, 1980). Two supportive studies were also included.

In the NTP-study, BMP induced treatment-related tumours at multiple sites in rats and mice (relevant Tables below). General toxicity consisted of decreased body weight in the rats, an average reduction of up to 14% in the two male high dose groups relative to controls, and decreased survival in the mid and high dose male and female rats as well as high dose male and female mice, primarily related to the presence of treatment-related neoplasms (preterm sacrifice).

In male rats, dose-related increases of benign and malignant tumours in a number of tissues were observed following exposure to BMP for 2 years - skin, subcutaneous tissue, Zymbal's gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, urinary bladder, lung and seminal vesicles, (Table below). The stop-exposure group showed that BMP dosing for only 3 months induced tumours at most sites where tumours were observed following 2-year exposure, in some tissues to a greater extent. The increase in subcutaneous neoplasms

were the primary reason for preterm sacrifice of moribund rats in the high-dose groups. In a number of other tissues, increased but not clearly dose-dependent incidences of tumours were observed - mammary gland, thyroid gland and the haematopoietic system, which were outside the range of the HCD. Increased incidences of adenomas in the kidney and pancreas were observed in the exposed groups as compared to controls, however these were within the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in male F344/N Rats (n=51-60/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996).

Site	Dose (mg/kg bw/day)					HCD
	0	100	200	430	800#	
Skin (adenoma, papilloma, carcinoma)	8	11	27**	44**	35**	2-16
Subcutaneous tissue (fibroma, fibrosarcoma, sarcoma)	4	17*	25**	29**	17**	0-16
Mammary gland (adenoma, fibroadenoma)	0	8*	14**	13**	8**	0-12
Zymbal's gland (adenoma, carcinoma)	4	2	8	9	25**	0-4
Oral cavity (papilloma, carcinoma)	0	8*	18**	18**	22**	0-4
Oesophagus (papilloma, carcinoma)	0	0	2	11*	0	N.A.
Forestomach (papilloma)	0	0	0	2	8	0-2
Small intestine (adenoma, carcinoma)	0	0	0	4	8*	0-4
Large intestine (adenoma, carcinoma)	0	0	6	7	17	0-2
Peritoneum (malignant mesothelioma)	0	6	16**	16**	43**	0-8
Kidney (renal tubule adenoma)	0	0	2	5**	2	0-6
Urinary bladder (papilloma, carcinoma)	0	0	2	5	3	0-2
Lung (adenoma, carcinoma)	2	2	6	7*	12*	0-8
Thyroid gland (adenoma, carcinoma)	0	4	12*	5	15**	0-6
Seminal vesicle (adenoma, carcinoma)	0	0	0	0	3	0
Haematopoietic system (mononuclear cell leukaemia)	53	55	78**	62**	42**	32-62
Pancreas (adenoma)	2	4	8*	6	5	0-10

**p<0.05 vs controls, **p<0.01 vs controls, N.A. = not available, #Dosed for 3 months and then maintained on a control diet.*

In female rats, dose-related increases in benign and malignant tumours in the oral cavity, oesophagus and thyroid gland were observed (Table below). In the mammary gland, increased, though not clearly dose-related, incidences of benign and malignant tumours were observed, which were outside the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in female F344/N Rats (n=50-52/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	115	230	460	
Oral cavity (papilloma, carcinoma)	4	6	9	12	0-6
Oesophagus (papilloma)	0	0	2	19**	N.A.
Mammary gland (fibroadenoma, adenoma, carcinoma)	54	92**	89**	90**	8-64
Thyroid gland (adenoma, carcinoma)	0	0	4	8**	0-6

***p<0.01 vs controls, N.A. = not available*

In male mice, dose-related increases of benign and malignant tumours in the Harderian gland, lung and forestomach were observed (Table below). Increased incidences of adenomas in the kidney outside the HCD were observed, although these were not clearly dose-related.

Table: Incidences (%) of selected treatment-related neoplasms in male B6C3F1 mice (n=49-51/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	35	70	140	
Harderian gland (adenoma, carcinoma)	8	14	32**	45**	0-20
Lung (adenoma, carcinoma)	30	22	32	51*	4-32
Forestomach (papilloma, carcinoma)	0	6	6	8*	0-6
Kidney (adenoma)	0	0	3	2	0-2

**p<0.05 vs controls, **p<0.01 vs controls*

In female mice, dose-related increases of benign and malignant tumours in the Harderian gland, lung, subcutaneous tissue and mammary gland were observed (Table below). Increased incidences of tumours in the mammary gland and circulatory system were observed compared to the controls, however these were within the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in female B6C3F1 mice (n=50-52/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	40	80	170	
Harderian gland (adenoma, carcinoma)	6	24**	25**	38**	0-10
Lung (adenoma, carcinoma)	10	10	29**	38**	2-26
Subcutaneous tissue (fibrosarcoma, sarcoma)	0	2	8	24**	0-8
Forestomach (papilloma)	0	2	10*	6*	0-14
Mammary gland (carcinoma)	0	0	2	6	0-10
Circulatory system (haemangioma, haemangiosarcoma)	2	4	0	10*	0-8

**p<0.05 vs controls, **p<0.01 vs controls*

Altogether, the outcome of this carcinogenicity study showed that exposure to FR-1138 caused multi-site tumours in both sexes of rats and mice without any overt signs of general toxicity, warranting classification as Carc. 1B. The stop-exposure group in male rats showed that BMP administered for only 3 months was carcinogenic and that the incidences of tumours in some tissues were greater than in the continuous-exposure groups.

In a follow-up study (Ton *et al.*, 2004) lungs from the B6C3F1 mice were characterized for mutations in the protooncogene K-ras. The frequency of mutated K-ras in BMP-induced lung neoplasms was significantly elevated over that of spontaneous lung neoplasms from control mice (57% vs 15%) and indicated that mutations in the K-ras gene could be involved in the observed lung carcinogenesis. However, RAC considered the result of this single study as providing limited mechanistic support for a genotoxic mechanism of action of BMP.

In the other 2-year carcinogenicity study in SD-rats performed by industry (Keyes *et al.*, 1980), no statistically significant treatment-related increases of tumour incidences were reported. However, only two doses were used, 5 and 100 mg/kg bw/day, which were considered low in comparison to the NTP study, with the high dose 100 mg/kg bw/day being equal to the low dose in the NTP study. In this study, the general toxicity was low, with only slight reductions in body weight in treated males and survival not significantly different among the groups. Thus, this study was given limited weight in the assessment.

The 90-day study (Elwell *et al.*, 1989) showed that kidney and bladder in both rats and mice are target organs for BMP, with renal papillary necrosis and renal tubular regeneration and fibrosis observed as well as urinary bladder hyperplasia. This study provided support to the effects seen in the NTP carcinogenicity study.

RAC conclusions

BMP induced dose-dependent multi-site tumours in two species, rats and mice, in a well-conducted OECD TG 453-study carried out by the NTP under GLP conditions and with limited general toxicity. Both benign and malignant tumours were observed in the respective tissues, showing the ability of the tumours to progress to malignancy. The stop-exposure group in male rats showed that only 3 months of exposure induced tumours at most sites where tumours were observed in the 2-year continuous-exposure groups. The incidences of neoplasms were greater at some sites (lungs, small and large intestine, thyroid). Adenoma and carcinoma of the seminal vesicle were also found, which did not occur in the other groups, and which are extremely rare in rats. Based on the findings from this group, genetic damage appears to occur within the first few months of exposure and that can develop into tumours, also in the absence of a toxic response in these tissues. Some of the tumours observed fit into the pattern of genotoxic chemicals (NTP, 1996).

One negative study in rats was included in the evaluation, however the study was conducted at doses similar to and lower than in the NTP-study and is therefore given less weight. Some support has also been provided on preneoplastic lesions in some target organs of a 90-day study, also indicating lack of preneoplastic lesions in many target tissues, and indications of genotoxicity were presented in a follow-up study on tissue material from the NTP-study.

Altogether, RAC consider BMP to be a multi-site carcinogen in two species with tumours of human relevance. Therefore, RAC agrees with the DS's proposal to classify BMP as **Carc. 1B; H350**.

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

Annex I to the 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: **-**

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CONTENTS

1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 1: Summary table of toxicokinetic studies

Method	***Results***	***Remarks***	***Reference***
Toxicokinetics study. *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, BMPD 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. The extensive metabolism of BMP after ingestion reduces the likelihood of systemic exposure to low levels.* *In summary, irrespective of the dose, route or duration of exposure, less than 5% of BMP was retained in the tissues. The predominat route of BMP elimination of BMP was urinary; irrespective of the route, concentration, nutritional state at onset or duration of exposure of BMP (>80% after 6h of exposure and increasing with time). BMP monoglucuronide was the only metabolite present in the urine of rats fed with BMP. Analysis for14C radioactivity in the liver and bile of rats treated with [14C[BMP] showed that over 50% of the dose was excreted in the bile within 6 h and over 99% of the BMP derived 14C equivalent excreted in bile over time consisted of glucuronide conjugate of BMP. BMP rapidly disappeared from the blood. The concentration-time profile of*	*Test substance:* *U-14C-labeled BMP* *Radioactively labeled BMP Purity: 97.3%* *Nonradiolabeled BMP purity: 98%* *Details of study:* *Animals:* *Conventional Male F344 rats* *Male F344 rats with indwelling jungular vein cannula (JVC)* *Male F344 rats with implanted bile duct cannulas* *Age at onset: 8-9 weeks* *Body weight at onset: 182-236 g* *Feeding: Animals were allowed food and water ab libitum except for a 12-h fasting period before a single administration of BMP. Food was returned 2 h after dosing. Animals used in the repeated oral administration studies were not fasted. Food was provided as a powder to reduce contamination of fecal matter* *Dose selection: Doses were selected based on historical data* *Subtoxic doses of 10, 100, 150, 300 and 600 mk/kg were chosen to assess the effect of dose on on the rate and route of excretion after oral gavage* *Does of 10 and 15 mg/kg were selected for the intravenous route of administration* *For repeated dose studies, 100 mg/kg was administered daily by oral gavage for 1, 5, amd 10 days*	(Hoehle *et al.*, 2009)

Method	***Results***	***Remarks***	***Reference***
	[14CBMP] after intravenous injection displayed a biexponential equation consistent with a two-compartment model with first order elimination. After a rapid initial distribution of [14C]BMP (theoretical half-life (t1/2α = 3.4 min), a significant slower elimination (t1/2β = 2 h). Blood plasma concentrations of BMP a later than 30 min were very low. Absorption of BMP was rapid after oral administration with Cmax reached after 40 min. 14C equivalents were detected in the blood, although at very low levels, throughout to termination at 72 h. Both parent and BMP glucuronide were detected in the blood plasm after both oral and intravenous exposure. BMP glucuronide concentration increased in the blood plasma over time. By Cmax (40 min after oral exposure), the majority of radioactivity in blood plasma was BMP glucuronide.	*Doses provided 25 to 200 µCi/kg [14C]BMP* *Number of animals: 4 animals/study with the exception of the BDC study in which 3 animals were used* *Vehicle: Cremophore EL-absolute ethanol-water (3:1:1, v/v/v)* *Sample collection and preparation after dosing:* *In single dose studies, urine was collected at 6, 12, 24, 36, 48, and 72 h while feces were collected at 12, 24, 36, 48 and 72 h* *In the reoeted dose studies, urine was collected at 6, 12, and 24 h after each dose, whereas feces were collected 12 and 24 h after administration* *Metabolic cages wre rinsed with methanol after the collection of urine. Radioactivity recovered in cage rinses was added to that determined for urine* *10-100µl of urine cage rinse and feces were analysed 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, somach, stomach content, skin, and testes) of animals were analysed by LSC to determine their total 14C content* *Biliary study dose was [14C]BMP (15 mg/kg; 25 µCi/kg; 1 mg/kg) administered by tail vein injection and bile was collected on ice at time points from 0.025 to 6 h. Bile samples were analysed by LSC and HPLC.* *Blood kinetics after intravenous and oral administration of BMP: For the determination of*	

Method	***Results***	***Remarks***	***Reference***
		intravenous blood kinetics [14C]BMP (10 or 15 mg/kg; 50 µCi/kg; 2 mg/kg) was administered intravenously through jugular vein cannulation (JVC) [in a solution of Cremophpore EL-absolute ethanol-water (3:1:1; v/v/v)]. For oral blood kinetics studies, [14C]BMP (10 mg/kg; 200 µCi/kg; 4 ml/kg) was administered by oral gavage to F-344 rats with JVcs. Blood samples (300 µl) were collected via the JVC at 0.083, 0.125, 0.25, 0.5, 1, 1.5, 3, 6, 9, 12, 24, 36, and 48 hinto heparinizedsyringes. Aliquots of these blood samples were solubilized and 14C radioactivity was quantified by LSC. [14C]BMP content in plasma from the blood samples were also determined by HPLC. *Aliquots (150µl) of each blood sample obtained atefr oral administration were extracted with ethyl acetate and the supernatant and precipitate from the supernatant subjected to HPLC with radiometric detection.* *Pharmacokinetic analysis: The blood concentration-time data after intravenous (parent BMP) and oral administration (total [14C]BMP and parent BMP) were analysed using a computer modelling program (WinNonlin Professional, version 5.1; Pharsight, Mountain View, CA) for the determination of the half-life of distribution (t1/2α), terminal half-life for elimination (t1/2β), and the maximum oral bioavailability.* *Identification of phase II metabolites: The presence of glucuronide and sulfate conjugates of BMP in urine were analysed for by HPLC* *Identification of 14C radioactivity in blood and liver: The identity of 14C equivalent in blood was*	

Method	***Results***	***Remarks***	***Reference***
		characterized total blood from rats after intravenous (2 F-344 rats; 10 mg/kg; 200μCi; I ml) or oral (4 F-344 rats; 10 mg/kg/; 200 μCi/kg; 2 ml) administration of BMP byt HPLC	
Toxicokinetics study. *Reliability index of study: 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 identified.* *[14C]BMP and non-labeled BMP were converted into monoglucuronide by hepatic microsomal proteins or primary hepatocytes obtained from male F-344 rats. The formation of BMP was stoichiometric with the loss of BMP, and it increased with increase in concentration of microsomal proteins and with time of incubation* *Kinetic analysis of BMP glucuronide formation using hepatic microsomes from male and female F-344 rats 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 presence of UDPGA did not convert BMP into a detectable amount of BMP glucuronide. The kinetics did not follow Michaelis-Menten kinetics. Human*	*Animals: Male F-344 rats* *Weight of animals: 200 – 325 g* *Microsomal fractions:* *Pooled microsomes were prepared from 8 male F-344 rats. In addition, Pooled liver microsomes from female F-344 rats (pool of 105), male B6C3F1 mice (pool of 347 animals), male Golden Syrian hamster (pool of 101 animals), male rhesus monkeys (pool of 12 animals), and pooled human liver microsomes for 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* *Rat and human hepatocyte preparation:* *Hepatocyes were prepared from unspecified number of male F-344 rats with a viability of >90% as determined by tryptan blus exclusion. Pooled cryopreserved human hepatocytes were prepared from 10 donors (5 males and 5 females of white and African Americans, with ages ranging from 20 to 74. Human hepatocytes has a viability of >70% as determined*	(Rad *et al.*, 2010)

Method	***Results***	***Remarks***	***Reference***
	microsomes converted TFMU into TFMU glucuronide; indicating that human micrososmes possessed glucuronidation activity. *Human intestinal microsomes converted BMP to BMP glucuronide at a slower rate than than human hepatic microsomes* *Only one (UGT2B7) of six expressed human hepatic UGTs incubated with [14C]BMP, actively converted BMP into BMP glucuronide; albeit, at a very slow rate* *When [14C]BMP was incubated with suspensions of human cryopreserved hepatocytes, BMP glucuronide was generated 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 rat hepatocytes was >150-fold higher than that of human hepatocytes; although human hepatocytes possesed glucuronidation activity as shown by their ability to convert [14C]BPA into glucuronide.* *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).*	*by tryptan blue exclusion* *ASSAYS* *1. Glucuronidationa assay: UDP-glucuronosyltransferase (UGT) enzymes activities toward BMP, was determined in the above listed microsomes* *Controls included incubation with TFMU, a known substrate for a number of UGT enzymes as well incubation with heat-denatured microsomes or with intact microsomes in the absence UPGA. For incubation with recombinant UGTs, the control incubation consisted of Supersomes lacking UGT enzymes, but in the presence of UDPGA.* *All incubations were performed in duplicate on at least three occasions* *For concentration-dependent metabolism studies, incubations were conducted with [14C]MBP at final concentrations of 3.5, 7, 15, 25, 50 100, 250, 500, or 1000 μM (0.2-0.9 μCi/ml) and rat liver microsoms (0.25 mg/ml). Glucuronidation activities for each substrate concentration were determined in three independent experiments in duplicate* *Hepatocyte incubations:* *Rat hepatocytes (0.25-1 x 106 cells/ml) were incubated in suspension with WEM and [14C]BMP (2, 25, 50,75, or 100 μM; 0.2-0.6μCi/ml, 0.25% DMSO-absolute ethanol; 10:1) for 120 min* *Human hepatocytes (0.25 x 106 cells/ml) were incubated as rats hepatocytes with [14C]BMP (2, 25, or 50 μM; 0.2-0.6 μCi/ml) for 360 min* *Aliquots from the rat and human*	

Method	*Results*	*Remarks*	*Reference*
		hepatocyte incubations, respectively, were collected at various times and analysed by HPLC *Incubation with rat hepayocytes were conducted three times in duplicate for each BMP concentration* *[14C]BPA (50 µM, 0.2 µCi/ml) was included to verify glucuronidation activity of rat and human hepatocytes* *Negative control incubations were conducted using substrate in WEM without cells* *Identification of Phase II metabolites:* *For the identification of conjugates of BMP, samples were from microsomal and hepatocyte incubations were subjected to enzymatic hydrolysis by β-glucuronidase or sulfates followed by HPLC, liquid chromatography-MS and MS//MS analyses* *Data Analysis: The amount of glucuronide formed and glucuronidation activity were calculated from the area of the HPLC glucuronide peak using the specific activity of the [14C]BMP stock solution. For the kinetic studies, the data were subjected to analysis based on Michaelis-Menton kinetics with OriginPro 6.0 software (OriginLab Corporation, Northampton, MA)*	

1.1.1 Hoehle et al. (2009)

Study 1 reference:

Hoehle, S. I., et al. (2009). "Absorption, distribution, metabolism, and excretion of 2,2-bis(bromomethyl) -1,3-propanediol in male fischer-344 rats." Drug Metabolism and Disposition **37**(2): 408-416.

(Key study in dossier)

Test type

Absorption, Distribution, Metabolism, and Excretion study (ADME) Similar to OECD TG 417. No GLP.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* The test substance is equivalent to the substance identified in the dossier
- *Degree of purity:* 97.3% pure radiolabelled BMP and 98% pure nonlabeled BMP
- *Impurities (or a note that the impurities do not affect the classification):* Negligible impurities that did not affect the classification
- *Batch number:* Lot 10426-17-34
- *Physicochemical properties that may be important when assessing toxicokinetics*

  U-14C labeled BMP, in absolute ethanol (1mCi/ml) was obtained from Midwest Research Institute (Kansas City, MO). The radiochemical purity of BMP was determined by reverse-phase HPLC-UV/visible-radiometric analysis to be 97.3%. The specific activity was reported to be 65.1mCi/mmol (247uCi/mg). Non radiolabeled BMP was obtained from Sigma-Aldrich (St. Louis, MO). Chemical purity of the unlabeled was 98%.

Test animals

- *Species/strain/sex:* Male F-344 rats
- *No. of animals per sex per dose: 4 animals/study with the exception of the BDC study in which 3 animals were used*
- *Age and weight at the study initiation: 9 weeks old (182-236g)*

Administration/exposure

- *Route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other : Oral gavage and intravenous through jugular vein catheter*
- *duration of test/exposure period: 1, 5 or 10 days*
- *doses/concentration levels, rationale for dose level selection: Doses were selected based on historical data*

  Subtoxic single doses of 10, 100, 150, 300 and 600 mg/kg bw by oral gavage, 10 and 15 mg/kg for intravenous route. Repeat oral doses were 100 m/kg bw/day.
- *frequency of treatment: Once a day either by oral gavage or one single dose intravenously (jugular vein catheter)*
- *control group and treatment: No controls reported.*
- *historical control data: Not presented*
- *post exposure observation period: 6, 12, 24, 36, 48 and 72 hours*

- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water: Cremophore EL-absolute alcohol (3:1:1, v/v/v). BMP is soluble in the vehicle and also, the vehicle is not toxic to rats.*

- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:*

- *actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test: Doses provided 25 to 200 μCi/kg [^{14}C] BMP*

Results and discussion

Irrespective of the dose, route or duration of exposure, less than 5% of BMP was retained in the tissues. The predominant route of BMP elimination of oral or intravenous BMP was urinary; irrespective of the route, concentration, nutritional state at onset or duration of exposure of BMP (>80% after 6h of exposure and increasing with time). BMP monoglucuronide was the only metabolite present in the urine of rats fed with BMP. Analysis for ^{14}C radioactivity in the liver and bile of rats treated with [^{14}C] -BMPshowed that over 50% of the dose was excreted in the bile within 6 h and over 99% of the BMP derived ^{14}C equivalent excreted in bile over time consisted of glucuronide conjugate of BMP. It underwent enterohepatic recycling with subsequent elimination in the urine. BMP rapidly disappeared from the blood. The concentration-time profile of [^{14}C] -BMPafter intravenous injection displayed a biexponential equation consistent with a two-compartment model with first order elimination. After a rapid initial distribution of [^{14}C]-BMP (theoretical half-life ($t_{1/2\alpha}$ = 3.4 min), a significant slower elimination ($t_{1/2\beta}$ = 2 h). Blood plasma concentrations of BMP at later than 30 min were very low. Absorption of BMP was rapid after oral administration with C_{max} reached after 40 min. ^{14}C equivalents were detected in the blood, although at very low levels. Both parent and BMP glucuronide were detected in the blood plasm after both oral and intravenous exposure. BMP glucuronide concentration increased in the blood plasma over time. By C_{max} (40 min after oral exposure), the majority of radioactivity in blood plasma was BMP glucuronide.

The total amt. of radioactivity remaining in tissues at 72 h after a single oral administration of BMP (100 mg/kg) was less than 1% of the dose, and repeated daily dosing did not lead to retention in tissues. In all studies, the radioactivity recovered in feces was low (<15%).

Conclusion: BMP was rapidly absorbed from the GI tract into the portal circulation and efficiently metabolized in the liver to a glucuronide conjugate that eventually was excreted in the urine of rats. The extensive excretion and rapid glucuronidation by the liver may limits exposure of internal tissues to BMP by greatly reducing its systemic bioavailability after oral exposure.

Table 2: Percentage of dose recovered from tissues and excreta 12 hr after last oral adminstration

TABLE I

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.

1.1.2 Rad et al. (2010)

Study reference:

Rad, G., Hoehle S.I., Kuester R. K, Glenn Sipes I. In vitro glucuronidation of 2,2-Bis(bromomethyl)-1,3-propanediol by microsomes and hepatocytes from rats and humans. Drug Metabolism and Disposition **38**(6): 957-962. 2010

Test type: Absorption, Distribution, Metabolism, and Excretion study (ADME. OECD TG 417)

Detailed study summary and results:

Test type

Description of the test design.

- *number of replicates:* 2
- *number of doses, justification of dose selection*: 9 dose for assays with microsomes, 5 doses for assays with rat hepatocytes and 3 doses for assays with human hepatocytes
- *positive and negative control groups and treatment:* Controls included incubation with TFMU, a known substrate for a number of UGT enzymes as well incubation with heat-denatured microsomes or with intact microsomes in the absence UPGA. For incubation with recombinant UGTs, the control

incubation consisted of Supersomes lacking UGT enzymes, but in the presence of UDPGA. [14C]-BPA (50 μM, 0.2 μCi/ml) was included in the assays with hepatocytes to verify glucuronidation activity of rat and human hepatocytes. Negative control incubations were conducted using substrate in WEM without cells

- *details on slide preparation: Not applicable*
- *number of metaphases analysed: Not applicable*
- *justification for choice of vehicle:* [U-14C]BMP and nonlabeled BMP were dissolved in absolute ethanol (1 mCi/ml)
- *solubility and stability of the test substance in vehicle not reported*
- *description of follow up repeat study:* Incubation with rat hepatocytes were conducted three times in duplicate for each BMP concentration. For the identification of conjugates of BMP, samples from microsomal and hepatocyte incubations were subjected to enzymatic hydrolysis by β-glucuronidase or sulfates followed by HPLC, liquid chromatography-MS and MS//MS analyses
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):* The amount of glucuronide formed and glucuronidation activity were calculated from the area of the HPLC glucuronide peak using the specific activity of the [14C]-BMP stock solution. For the kinetic studies, the data were subjected to analysis based on Michaelis-Menton kinetics with OriginPro 6.0 software (OriginLab Corporation, Northampton, MA)
- *Please state if the study is GLP compliant or not:* Not stated

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* The test substance is equivalent to the substance identified in the CLH dossier
- *Degree of purity:* 97.3% (radioactive labelled BMP) and 98% (nonlabeled BMP)
- *Impurities (or a note that the impurities do not affect the classification*): The impurities do not affect the classification
- *Batch number:* lot 10426-17-34, identical substance as in the previous study in 2.1.1.

Administration/exposure

Strain or cell type or cell line, target gene if applicable: Rat (male F344, HSD, Indianapolis) and human hepatocytes (CellzDirect, Austin, TX) ; liver microsomes from rats, rhesus monkeys and intestinal and liver microsomes from humans (XenoTech, LLC); supersomes from insect sf-9 cells infected with a baculovirus strain containing the cDNA of human UGT1A1, 1A3, 1A4, 1A6, 1A9, or 2B7 (BD Gentest).

Type and composition of metabolic activation system:

Test concentrations, and reasoning for selection of doses if applicable:

Microsomes: For concentration-dependent metabolism studies, incubations were conducted with [^{14}C]-MBP at final concentrations of 3.5, 7, 15, 25, 50 100, 250, 500, or 1000 μM (0.2-0.9 μCi/ml) and rat liver microsoms (0.25 mg/ml).

Hepatocytes: Rat hepatocytes (0.25-1 x 106 cells/ml) were incubated in suspension with WEM and [^{14}C]-BMP (2, 25, 50, 75, or 100 μM; 0.2-0.6μCi/ml, 0.25% DMSO-absolute ethanol; 10:1) for 120 min. Human hepatocytes (0.25 x 106 cells/ml) were incubated as rats hepatocytes with [14C]BMP (2, 25, or 50 μM; 0.2-0.6 μCi/ml) for 360 min.

[^{14}C]-BPA (50 μM, 0.2 μCi/ml) was included to verify glucuronidation activity of rat and human hepatocytes

Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water): BMP was dissolved in absolute ethanol and diluted to the various concentrations with water

Statistical methods: Data Analysis: The amount of glucuronide formed and glucuronidation activity were calculated from the area of the HPLC glucuronide peak using the specific activity of the [^{14}C]-BMP stock solution. For the kinetic studies, the data were subjected to analysis based on Michaelis-Menton kinetics with OriginPro 6.0 software (OriginLab Corporation, Northampton, MA) Co-factors used: Alamethicin (5μg/ml)

Results and discussion

In this study, the in vitro hepatic glucuronidation of BMP was compared across several species. In addition, the glucuronidation activities of human intestinal microsomes and specific human hepatic UDP-glucuronosyltransferase (UGT) enzymes for BMP were determined. The results showed that [^{14}C]-BMP and non-labeled BMP were converted into monoglucuronide by hepatic microsomal proteins or primary hepatocytes obtained from male F-344 rats. The formation of BMP was stoichiometric with the loss of BMP, and it increased with increase in concentration of microsomal proteins and with time of incubation

Kinetic analysis of BMP glucuronide formation using hepatic microsomes from male and female F-344 rats 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 presence of UDPGA did not convert BMP into a detectable amount of BMP glucuronide. The kinetics did not follow Michaelis-Menten kinetics. Human microsomes converted TFMU into TFMU glucuronide; indicating that human micrososmes possessed glucuronidation activity.

Human intestinal microsomes converted BMP to BMP glucuronide at a slower rate than human hepatic microsomes

Only one (UGT2B7) of six expressed human hepatic UGTs incubated with [14C]BMP, actively converted BMP into BMP glucuronide; albeit, at a very slow rate

When [^{14}C]-BMP was incubated with suspensions of human cryopreserved hepatocytes, BMP glucuronide was generated 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 human hepatocytes was < 150-fold than that of F-344 hepatocytes; although human hepatocytes possess glucuronidation activity as shown by their ability to convert [^{14}C]-BPA into glucuronide

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).The authors discuss that the BMP seems to behave similarly to aliphatic alcohols (C5–C7) with respect to differing glucuronidation affinities in rat and human liver microsomes, a finding in referred studies.

Conclusion: Glucurodination is the sole route of metabolism of BMP in liver microsomes or primary liver cells of rodents, Rhesus monkey and human. Rodents displayed the highest glucurodination capacity. The poor glucurodination capacity of BMP by humans suggests that its in vivo pharmacokinetic profile will differ dramatically from that obtained in rodents

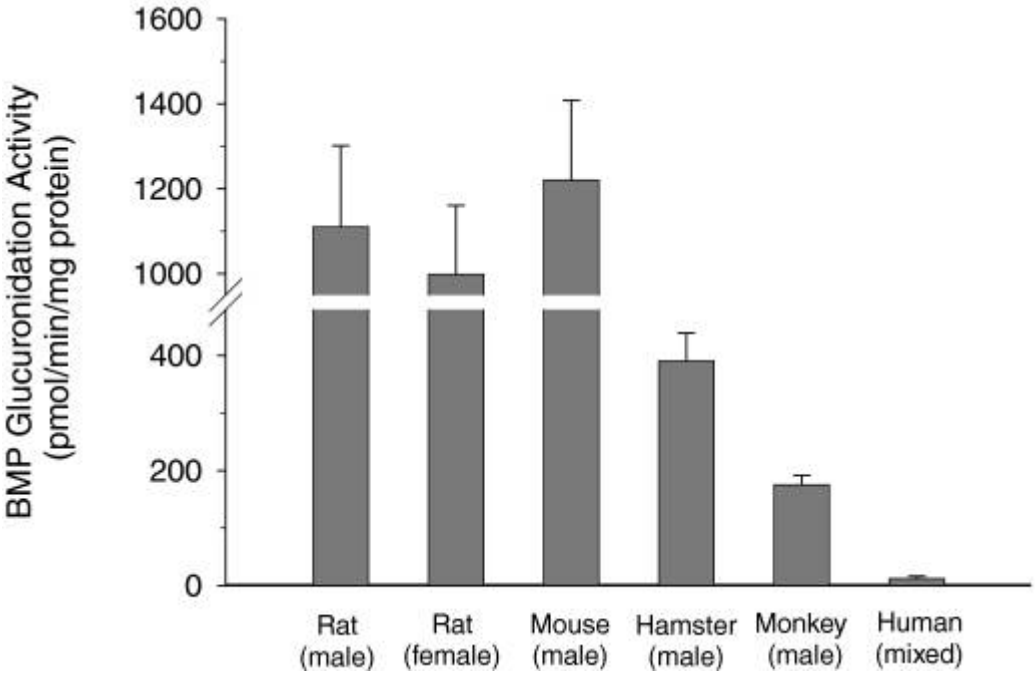

Figure 1: Activities of hepatic microsomes from male and female F-344 rats, male B6C3F1 mice, male Golden Syrian hamsters, male rhesus monkeys, and humans (mixed gender) for the glucuronidation of BMP. Rates of glucuronidation were determined at 50 µM BMP and are expressed as picomoles per minute per milligram of protein (mean ± S.D. of at least three independent experiments). Adopted from: Rad G, Hoehle SI, Kuester RK, Sipes IG (2010). In vitro glucuronidation of 2,2-bis(bromomethyl)-1,3-propanediol by microsomes and hepatocytes from rats and humans. Drug Metab Dispos. 2010 Jun;38(6):957-62. doi: 10.1124/dmd.110.032110. Epub 2010 Mar 3.

2 HEALTH HAZARDS

2.1 Germ cell mutagenicity

2.1.1 In vitro data

2.1.1.1 Unknown author (1996)

Study reference:

Unknown author (1996). http://echa.europa.eu/registration-dossier/-/registered-dossier/7873/7/7/2

NOTE: The two studies in the dossier are performed and evaluated in the same way, and reported identically.

The most noticeable differences between the studies is that different batches of FR-522 with different purity are used. The results and conclusions are identical.

Detailed study summary and results:

Test type

TG guideline: OECD Guideline 471 (Bacterial Reverse Mutation Assay) US EPA, Method: HG-Gene MUta-S. typhimurium; the Salmonella typhimurium reverse mutation assay, 1984

- *number of replicates: 3*

  number of doses, justification of dose selection: 4 doses.

  These doses were selected on the basis of the results that were obtained from a preliminary toxicity test (Preliminary toxicity test: 5, 50, 500, 5000 mg/plate) which showed that BMP is cytotoxic in the presence or absence of liver S-9/Hamster S-9 mix at the concentrations >5000 µg/plate in the bacterial reverse mutation assay
- *positive and negative control groups and treatment:* The positive control was N-ethyl-N-nitrosoguanidine while DMSO was applied as the negative control
- *justification for choice of vehicle:* BMP was dissolved in DMSO. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity
- *solubility and stability of the test substance in vehicle if known:* Fresh BMP solution and dilutions were prepared in DMSO for the assay
- *description of follow up repeat study:* All positive results were reproduced.
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*

  1. Mutagenic response- a dose-related, reproducible increase in the number of reverbants over background

  2. Nonmutagenic reponse: When no increase in revertants was elicited by the chemical

  3. Questionable response: when there was an absence of clear-cut dose related increase in revertants; when the dose-related increases in the number of revertants were not reproducible; or when the response was of insufficient magnitude to support a determination of mutagenicity.
- *C*ompliant with GLP and OECD guideline 471

Test substance

The test material is BMP which is equivalent to the substance identified in the CLH dossier. There is ample impurities (insignificant) in the substance and as such do not affect the classification. The following properties of the substance are described in the report:

Name of substance: FR522 (2,2-Bis(bromomethyl)-1,3-propanediol

CAS number: 3296-90-0

EC number: 221-967-7

Purity: 98.63,

Batch no. Not specified

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable*

Bacteria strains: Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537

Type and composition of metabolic activation system: Metabolic activation: 10-30% S9-mix prepared from rat liver cells induced with Aroclor or; S9-mix prepared from hamster liver cells without Aroclor induction

- *Test concentrations, and reasoning for selection of doses if applicable*

  BMP mutation test concentrations: 0, 50, 150, 500, 1500, 5000 µg/plate.

  The main test doses were based on the initial toxicity test in the presence or absence of S9 which defined a >5000 µg/plate of BMP as cytotoxic in the bacterial reverse mutation assay

- *Vehicle:* The vehicle was DMSO. BMP is more soluble in DMSO than in water. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity. The volume of DMSO used in the study was in conformity with the recommendation of OECD test guideline 471.

Results and discussion

The preliminary cytotoxicity test performed with S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 strains were negative up to the concentration of 5000 µg/plate BMP in absence and presence of S9. Cytotoxicity was observed at >5000 µg/plate of BMP. Therefore, further mutations tests were terminated at this initial cytotoxicity concentration of 5000 µg/plate. Main mutation test performed with S. typhimurium, TA 1537 and TA 98 strains were negative in the presence or absence of S9 while S. typhimurium TA100 and TA 1535 displayed positive genotoxicity in presence of hamster S9. This effect was dependent on the hamster S9 concentration. The highest effect was observed in the presence of the highest concentration of 30% hamster S9. Five positive controls were also included in the test. Among these, three (N-ethyl-nitro-N-nitrosoguanidine, 9-aminoacridine and 2-nitrofluorene, were tested in the absence of S9 while two (2-Aminoanthracene, and Congo red) were tested in the presence of S9. The negative control was DMSO solvent vehicle. Three positive control test substances (N-ethyl-N-nitro-N-nitroguanidine, 9-aminoacridoine and 2-nitrofluorene) were tested in the absence of S9. In addition, two positive control substances (2-Aminoanthracene and congo red) were tested in the presence of S9.

In the main test, BMP was genotoxic in S. typhimurium TA and TA 1535 strain in the presence of hamster S9. These effects were dose dependent peaking at 30% S9 concentration in the assays. The means of the frequencies of reversion showed significant differences between the test substances and negative controls across the respective replicates. The other bacteria strains (TA 1537 and TA 98) not did display increase in revertant colonies when exposed to BMP in the absence or presence of S9.

In conclusion, mutagenic activity was not evident when BMP was tested in DMSO in the presence or absence of rat S9. On the other hand, BMP shows a clear evidence of mutagenic activity with strain TA 1535 and TA 100 in the presence of hamster S9.

2.1.1.2 Zeiger et al. (1992)

Study reference:

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from-the testing of 311 chemicals. Environ. Mol. Mutagen. 19 (Suppl. 21), 2-141.

Detailed study summary and results:

This is a summary paper

Test type

TG guideline: OECD Guideline 471 (Bacterial Reverse Mutation Assay)

- *number of replicates: 3*

  number of doses, justification of dose selection: 5

  BMP was run initially in a toxicity assay to determine the appropriate dose range for the mutagenicity assay. The toxicity assay was performed using TA100. Toxic concentrations were defined as those that produced a decrease in the number of colonies, or a clearing in the density of the background lawn, or both. BMP was tested in half-log dose intervals up to a dose to elicited toxicity
- *positive and negative control groups and treatment*: The positive controls in the absence of S9 were treated with sodium azide for TA100 and 4-nitro-o-phenylenediamine for TA9, respectively. The negative controls were treated with congo red.
- *details on slide preparation: number of metaphases analysed:* Does not apply
- *justification for choice of vehicle: solubility and stability of the test substance in vehicle if known: description of follow up repeat study*: BMP was dissolved in DMSO. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*

  1. Mutagenic response- a dose-related, reproducible increase in the number of reverbants over background

  2. A chemical was designated non-mutagenic only after it had been tested in strains TA98, TA100, TA1535, and TA97 and/or TA1537, without activation and with 10% S9 and 30% rat and hamster S9. Occasionally, 5% S9 was used in addition to the 10% and 30% S9 to clarify equivocal or weak positive responses.
- *solubility and stability of the test substance in vehicle if known*: Fresh BMP solution and dilutions were prepared in DMSO for the assay
- *description of follow up repeat study:* All positive results were reproduced.
- The study is compliant with GLP and OECD guideline 471

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier*

  The following properties of the substance are described in the report:

  (2,2-Bis(bromomethyl)-1,3-propanediol

  CAS number: 3296-90-0

  Purity: 84%

  Batch no.: Not specified in the report

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable*
    - Salmonella typhimurium strains TA98 and TA100
- *Type and composition of metabolic activation system:* Metabolic activation: 10-30% S9-mix prepared from male rat liver cells induced with Aroclor or; S9-mix prepared from hamster liver cells without Aroclor induction
- *Test concentrations, and reasoning for selection of doses if applicable*
- *Vehicle:*

  BMP mutation test concentrations: 0, 10, 33, 100, 333, 1666, 3333, 6666 µg/plate.

  The main test doses were based on the initial toxicity test in the presence or absence of S9 which defined a >3333 µg/plate of BMP as cytotoxic in the bacterial reverse mutation assay
- *identification, concentration and volume used, justification of choice of vehicle (if other than water)*

  The vehicle was DMSO. BMP is more soluble in DMSO than in water. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity. The volume of DMSO used in the study was in conformity with the recommendation of OECD test guideline 471. Statistical analysis data are not presented in this report.

Results and discussion

BMP was found to be genotoxic for TA100 cells in the presence of 30% hamster S9 only. The increase in the numbers of his+ revertant colonies increased with the increased in the concentration of BMP in the assay.

2.1.1.3 Mortelmans et al. (1986)

Study reference:

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8 (Suppl. 7), 1-119.

Detailed study summary and results:

Test type

TG guideline: OECD Guideline 471 (Bacterial Reverse Mutation Assay)

- *number of replicates: 3*

  number of doses, justification of dose selection: 5

  BMP was run initially in a toxicity assay to determine the appropriate dose range for the mutagenicity assay. The toxicity assay was performed using TA100. The upper limit for the test chemical was 10 mg/plate in the initial toxicity assay. Toxic concentrations were defined as those that produced a decrease in the number of colonies , or a clearing in the density of the background lawn, or both. As a rule, at least one toxic dose was incorporated into the mutagenicity test.
- *Positive and negative control groups and treatment:* The following mutagens were used as concurrent positive controls: sodium azide for TA1535 and TA100, 4 nitro-o-phenylenediamine for

TA98, and 9-aminoacridine for TA97 and TA1537. Solvent treated cultures served as the negative controls

- *justification for choice of vehicle: solubility and stability of the test substance in vehicle if known: description of follow up repeat study:* BMP was dissolved in DMSO. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*

  1. Mutagenic response- a dose-related, reproducible increase in the number of revertants over background

  2, A chemical was designated non-mutagenic only after it had been tested in strains TA98, TA100, TA1535, and TA97 and/or TA1537, without activation and with 10% S9 and 30% rat and hamster S9. Occasionally, 5% S9 was used in addition to the 10% and 30% S9 to clarify equivocal or weak positive responses.
- *solubility and stability of the test substance in vehicle if known*: Fresh BMP solution and dilutions were prepared in DMSO for the assay
- *description of follow up repeat study:* Not applicable.
- The study is compliant with GLP and OECD guideline 471

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier*

  The following properties of the substance are described in the report:

  (2,2-Bis(bromomethyl)-1,3-propanediol

  CAS number: 3296-90-0

  Purity: 96.3%

  Batch no.: Not specified in the report
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable*

  Salmonella typhimurium strains TA98, TA1535, TA1537 and TA100
- *Type and composition of metabolic activation system:* Metabolic activation: 10% S9-mix prepared from male rat liver cells induced with Aroclor or; S9-mix prepared from hamster liver cells without Aroclor induction

  BMP mutation test concentrations: 0, 10, 33, 100, 333, 1000, 3333, 10 000 µg/plate.

The main test doses were based on the initial toxicity test in the presence or absence of S9 which defined a >3333 µg/plate of BMP as cytotoxic in the bacterial reverse mutation assay

- *identification, concentration and volume used, justification of choice of vehicle (if other than water)*

  The vehicle was DMSO. BMP is more soluble in DMSO than in water. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity. The volume of DMSO used in the study was in conformity with the recommendation of OECD test guideline 471. Statistical analysis data are not presented in this report.

Results and discussion

BMP was found to be non-genotoxic for TA100, TA1535, TA1537 and TA98 strains, respectively, in the presence of 10% rat or hamster S9. There was no significant difference in the number of revertant colonies in all the bacterial strains tested under the conditions specified in the report.

2.1.1.4 Galloway et al. (1987)

Study reference:

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Mol. Mutagen. 10 (Suppl. 10), 1-175.

NTP, 1996. Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138) (CAS no. 3296-90-0) in F344/N Rats and B6C3F1

Detailed study summary and results:

Test type

TG guideline: **In vitro mammalian chromosomal aberration test** (Test performed before OECD TG 473, 20.09.2014)

- *number of replicates:* 3
- *number of doses, justification of dose selection:*. Range of doses spanning four to five orders of magnitude, in half log increments.
- *positive and negative control groups and treatment:* The following mutagens were used as concurrent positive controls: triethylenemelamine, mitomycin C or cyclophosphamide. Solvent treated cultures served as the negative controls.
- *details on slide preparation: number of metaphases analysed:* For scoring SCEs, slides were stained for 10 minutes in concentrated Hoechst 33258 (5 µg/ml in pH 6.8 buffer) and exposure to black light at 55-60°C for about 5 minutes prior to Giemsa staining. For scoring ABS, slides were stained with Giemsa.

- *justification for choice of vehicle: solubility and stability of the test substance in vehicle if known: description of follow up repeat study:* BMP was dissolved in DMSO. DMSO does not react with BMP and is compatible with the survival of the CHO cells
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*

  The analyses examined the evidence for a dose relation and the absolute increase over the solvent control at each dose.

  Sister chromatid exchange (SCE): 50 cells per dose were scored from the three highest doses at which sufficient M2 cells were available, from a solvent control and from a weak positive control treated with low dose of mitomycin C (without S9) or cyclophosphamide (with S9).

  Aberration test: 100 cells were scored from each of the three highest dose groups having sufficient metaphases for analyses and from positive (triethylenemelanine, mitocyn C,, or Cyclophospahmide) and solvent controls.

  The initial dose selection for SCE assays was based on a preliminary growth inhibition test in which cells that excluded trypan blue were counted 24 hr after treatment with BMP. The top dose that was selected for cytogenetics assay was the dose that was estimated to reduce growth by 50%. Observations on cell growth and cell kinetics from the SCE were used to select doses and fixation times for the chromosome aberration tests.
- *solubility and stability of the test substance in vehicle if known:* Fresh BMP solution and dilutions were prepared in DMSO for the assay
- *description of follow up repeat study:* Dose selection for repeat trials involved a range of doses based on observations from the first trial
- The study is compliant with GLP and OECD guideline 471

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier*

  The following properties of the substance are described in the report:

  2,2-Bis(bromomethyl)-1,3-propanediol

  CAS number: 3296-90-0

  Purity: Not specified

  Batch no.: Not specified in the report

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable*

  Chinese Hamster Ovary Cells
- *Type and composition of metabolic activation system:* Metabolic activation: 10% S9-mix prepared from male rat liver cells induced with Aroclor
- *Test concentrations, and reasoning for selection of doses if applicable*

The dose range without S9 was 16.7-167 μg/ml, and 800-1200 μg/ml with S9 for the SCE assay. The dose range for aberration test (ABS) was 400-600 μg/ml without S9 and 1000 μg/ml with S9.

Initially, dose selection was based on a preliminary growth inhibition test in which cells that excluded trypan blue were counted 24 hr after treatment. The top dose was selected for the cytogenetic assays were those estimated to reduce growth by 50%. In addition, observations on cell growth and cell kinetics from SCE test were used to select doses and fixation times for chromosome aberration tests.

- *Vehicle:* DMSO
- *identification, concentration and volume used, justification of choice of vehicle (if other than water)*

  The vehicle was DMSO. BMP is more soluble in DMSO than in water. DMSO does not react with BMP and is compatible with the survival of the bacteria and the S9 activity. The volume of DMSO used in the study was in conformity with the recommendation of OECD test guideline 471.

- *Statistical methods*

  For SCEs data, a linear regression test (trend test) of SCEs per chromosome vs log of the dose was used. For individual doses, absolute increase in SCEs per chromosome of 20% or more over the solvent control was considered significant. For ABSs, linear regression analysis of the percentage of cells with aberrations vs log-dose was used for trend test. To examine absolute increases over control levels at each dose, a binomial sampling assumption was used. The P values were adjusted by Dunett's method into account the multiple dose comparisons. For data analysis, the total aberration category and the criterion for a positive response was that the adjusted P value be ≤ 0.05.

Results and discussion

In the SCE test without S9, no increase was found even at doses that induced toxicity and marked cell cycle delay. A very slight increase in SCEs occurred at toxic levels with S9. The top dose, 1.2 mg/ml, reduced confluence by about 75%. The aberration test without S9 was negative up to toxic levels even an extended fixation time of 20.5 hr. With S9 the aberration test was positive, partly because of breaks at the long arm of X chromosome. This effect is reported to persist to highly toxic levels.

Summary in the NTP report, only genotoxic data

Reference:
NTP (1996). NTP Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138(R)) (CAS No. 3296-90-0) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**:** 1-465.

Page 77 (photocopy, Figure)

To summarise the data from the publications from the NTP publications, the page from the final report may be clarifying, see Figure 2.

GENETIC TOXICOLOGY

2,2-Bis(bromomethyl)-1,3-propanediol was shown to be mutagenic *in vitro* and *in vivo*, but the conditions required to observe the positive responses were highly specific, and 2,2-bis(bromomethyl)-1,3-propanediol was not active in all assays. In the two *Salmonella* assays reported here (Table E1), 2,2-bis(bromomethyl)-1,3-propanediol gave a positive response only in the second assay (Zeiger *et al.*, 1992), which used a different concentration of S9 from the first assay (Mortelmans *et al.*, 1986). Metabolic activation, specifically in the form of 30% Aroclor 1254-induced male Syrian hamster liver S9, was required to obtain the mutagenic response; 10% hamster S9 was ineffective, as was 10% or 30% S9 derived from livers of pretreated rats. No other *Salmonella* strain/activation combination was responsive to the effects of 2,2-bis(bromomethyl)-1,3-propanediol.

In cytogenetic tests with cultured Chinese hamster ovary cells (Galloway *et al.*, 1987), 2,2-bis(bromomethyl)-1,3-propanediol did not induce sister chromatid exchanges, with or without S9 (Table E2), but a dose-related increase in chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated in the presence of induced rat liver S9 (Table E3). Both tests were conducted up to doses which induced marked cytotoxicity; cell confluence in the sister chromatid exchange test was reduced 75% at the top dose tested with S9 (1,200 μg/mL). A majority of the breaks which were observed in the aberration assay were located in the heterochromatic region of the long arm of the X chromosome. The reason for this preferential breakage site is not known. Also, the type of damage pattern seen with 2,2-bis(bromomethyl)-1,3-propanediol (induction of chromosomal aberrations but not sister chromatid exchanges) is unusual. Most chemicals which induce chromosomal aberrations also induce sister chromatid exchanges (Galloway *et al.*, 1987).

2,2-Bis(bromomethyl)-1,3-propanediol was also shown to be genotoxic *in vivo*. Significant increases in micronucleated normochromatic erythrocytes were observed in peripheral blood samples obtained from male and female mice exposed for 13 weeks to 2,2-bis(bromomethyl)-1,3-propanediol in feed (Table E6). These increases were observed in the two highest dose groups of male mice (5,000 and 10,000 ppm) and the three highest dose groups of female mice (2,500, 5,000, and 10,000 ppm).

In the first of two mouse bone marrow micronucleus tests performed to confirm the positive results seen in the 13-week feed study, inconsistent results were obtained between two trials which used the same dose range of 100 to 400 mg/kg 2,2-bis(bromomethyl)-1,3-propanediol, administered by gavage three times at 24-hour intervals (Table E4). Results of the first trial were negative; however, in the second trial, 2,2-bis(bromomethyl)-1,3-propanediol produced a clear, dose-related increase in micronucleated polychromatic erythrocytes. Because the positive response was not reproduced, the results were concluded to be equivocal.

In an attempt to clarify the results obtained in the first bone marrow micronucleus test, a second investigation was performed using both male and female mice. 2,2-Bis(bromomethyl)-1,3-propanediol was administered as a single intraperitoneal injection (150 to 600 mg/kg) and bone marrow samples were taken 48 hours after dosing. The results of this experiment, shown in Table E5, provide evidence of the ability of 2,2-bis(bromomethyl)-1,3-propanediol to induce micronuclei in bone marrow cells of female mice. Although male mice in all three dose groups showed a two-fold increase in the frequency of micronucleated polychromatic erythrocytes, the trend test was not significant due to the similarity in the responses, and pairwise analyses were also insignificant. The response in female mice was somewhat stronger (2.5-fold increase over background, at the highest dose) and was directly related to increasing doses of 2,2-bis(bromomethyl)-1,3-propanediol. These results were consistent with the stronger response observed in female mice in the 13-week feed study (Table E4).

In conclusion, 2,2-bis(bromomethyl)-1,3-propanediol was genotoxic *in vitro* and *in vivo*, inducing gene mutations in *Salmonella* strain TA100, chromosomal aberrations in cultured Chinese hamster ovary cells, and micronuclei in erythrocytes of male and female mice. The *in vitro* responses required S9.

Figure 2: Summary on genotoxicity from the NTP (1996) report

2.1.1.5 Kong et al. (2011)

Study reference:

Kong, W., et al. (2011). "Induction of DNA damage in human urothelial cells by the brominated flame retardant 2,2-bis(bromomethyl)-1,3-propanediol: Role of oxidative stress." Toxicology 290(2-3): 272-278.

Detailed study summary and results:

Test type

- *number of replicates: 3*
- *number of doses, justification of dose selection:4*
- *positive and negative control groups and treatment:* The positive control groups were treated with either H2O2 or KBrO3. The negative control groups were treated with the vehicle (distilled water)

  details on slide preparation: Standard and modified comet assays were done using the Comet Assay hOGG1 FIARE Assay kits (Trevigen, Gaithersbutg, MD) as described by the kits manufacturers. Comet slides were analysed at 40 x magnifications under Olympus ImT-2 epiflouresecence microscope (Center Valley, PA) equipped with an excitation filter of 460-500 nm, a 100W mercury lamp, a long pass filter at 515 nm and a Hamamatsu Orca 100 digital camera (Bridgewater, NJ). The images were collected using Hamamatsu simple PCI digital imaging software program (V1.5, Tritek Corp. computer generated % tail DNA (T%DNA) was used to assess DNA damage. In the hIGG1 modified comet assay, the difference in the extent of DNA strand breaks (Net T%DNA between hoGG1–treated and buffer-treated control slides was determined to give a quantitative measurement of hOGG1 sensitive sites.
- *number of metaphases analysed*: For each exposure group, a total of 100 cells were analysed using systemic random sampling.
- *justification for choice of vehicle:* BMP was dissolved in distilled water. Distilled water does not react with BMP and is compatible with the survival of the CHO cells at the tested concentrations
- *solubility and stability of the test substance in vehicle if known:* BMP is soluble and stable in distilled water
- *description of follow up repeat :* All experiments were repeated at least three times.
- *Study criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*100 cells/dose were analysed for aberrations.
- No GLP

Test substance

The following properties of the substance are described in the report:

2,2-Bis(bromomethyl)-1,3-propanediol (BMP)

CAS number: 3296-90-0, EC number: 221-967-7

Purity: 98%

Batch nr. Not specified in the report

Test substance concentrations:

Preliminary cell viability test: 0, 250, 500, 750, 1000, 1250, 1500 µM

Mutation test: 0, 5, 10, 25, 100 µM

This test material used in the study is equivalent to the substance identified in the CLH dossier

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* Human Urothelial cells were exposed to BMP in this study. No metabolic activation was used in the assay
- *Vehicle:* distilled water
- *identification, concentration and volume used, justification of choice of vehicle (if other than water)*

  The vehicle was distilled water. BMP is more soluble in distilled water than in water. distilled water does not react with BMP and is compatible with the survival of the Urotsa cells.
- *Statistical methods*

  All data are presented as means±SEM. Data set were subjected to ANOVA (one-way or two-way) followed by Dunnetts's post-hoc analysis or unpaired t-test (GraphPad Prism, GraphPad Software, Inc.,). $p \leq 0.05$ was accepted as the level of significance.

Results and discussion

Initial Cell viability test was performed to determine the level of cytotoxicity that BMP may induced in URrotsa cells. This test showed that BMP was not cytotoxic in Urotsa cells. Over 90% of URotsa cells were viable upon exposure to BMP at the tested concentrations and conditions. Upon exposing URotsa cells to 25 µM BMP at various time points, a statistically significant increase in T% DNA was observed at 1 and 3h, respectively. No significant increase was observed at later time points. Since the DNA damage was observed early after BMP exposure, the concentration dependent effects of BMP exposure was investigated. Results indicated that after 1h of BMP treatment (5-100µM) induced a concentration dependent increase on T% DNA in URotsa cells. These BMP associated strand breaks were not persistent as evidenced by the return of the T% DNA to basal levels by 6h.

In the present study, using SV-40 immortalized human urothelial cells (UROtsa), endpoints associated with BMP induced DNA damage and oxidative stress were investigated. The effects of time (1-24. h) and concentration (5-100. muM) on BMP induced DNA strand breaks were assessed via the alkaline comet assay. The results revealed evidence of DNA strand breaks at 1 and 3. h following incubation of cells with non-cytotoxic concentrations of BMP. Strand breaks were not present after 6. h of incubation. Evidences for BMP associated oxidative stress include: an elevation of intracellular ROS formation as well as induction of Nrf2 and HSP70 protein levels. In addition, DNA strand breaks were attenuated when cells were pre-treated with . N-acetyl-. l-cysteine (NAC) and oxidative base modifications were revealed when a lesion specific endonuclease, human 8-hydroxyguanine DNA glycosylase 1 (hOGG1) was introduced into the comet assay. In conclusion, these results demonstrate that BMP induces DNA strand breaks and oxidative base damage in UROtsa cells. Oxidative stress is a significant, determinant factor in mediating these DNA lesions.

2.1.1.6 Kong et al. (2013)

Study reference:

Kong, W., et al. (2013). "Comparison of 2,2-bis(bromomethyl)-1,3-propanediol induced genotoxicity in UROtsa cells and primary rat hepatocytes: Relevance of metabolism and oxidative stress." Toxicology Letters 222(3): 273-279.

Detailed study summary and results:

Test type

- *number of replicates: 3*
- *number of doses, justification of dose selection:3*
- *Positive and negative control groups and treatment*: The positive control groups were treated with H2O2. The negative control groups were treated with the vehicle (5% Ethanol)
- *details on slide preparation:* Standard comet assays were done using the Comet Assay hOGG1 FIARE Assay kits (Trevigen, Gaithersbutg, MD) as described by the kits manufacturers. Comet slides were analysed at 40 x magnifications under Olympus ImT-2 epiflouresecence microscope (Center Valley, PA) equipped with an excitation filter of 460-500 nm, a 100W mercury lamp, a long pass filter at 515 nm and a Hamamatsu Orca 100 digital camera (Bridgewater, NJ). The images were collected using Hamamatsu simplePCI digital imaging software program (V1.5, Tritek Corp. computer generated % tail DNA (T%DNA) was used to assess DNA damage.
- *Number of metaphases analysed:* For each exposure group, a total of 100 cells were analysed using systemic random sampling.
- *Justification for choice of vehicle:* BMP was dissolved in 5% ethanol. Ethanol does not react with BMP and is compatible with the survival of the CHO cells at the tested concentrations
- *solubility and stability of the test substance in vehicle if known:* BMP is soluble and stable in 5% ethanol
- *Description of follow up repeat:* All experiments were repeated at least three times.
- *Study criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):*100 cells/dose were analysed for aberrations.
- No GLP

Test substance

The following properties of the substance are described in the report:

[14C-labeled)] 2,2-Bis(bromomethyl)-1,3-propanediol (BMP) and non-labelled BMP

CAS number: 3296-90-0

Labelled BMP Purity: 97.3%

Non-labelled BMP Purity: 98%

Batch nr.: Not specified in the report

This test material used in the study is equivalent to the substance identified in the CLH dossier

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* Human Urothelial cells and primary rat hepatocytes were exposed to BMP in this study. No metabolic activation was used in the assay
- *Test concentrations, and reasoning for selection of doses if applicable*

  Test substance concentration: 0, 25, 50, 100 μM
- *Vehicle:* 0.5% Ethanol
- *identification, concentration and volume used, justification of choice of vehicle (if other than water)*

  The vehicle was 5% ethanol. BMP is more soluble in 5% ethanol than in water. 5% ethanol does not react with BMP and is compatible with the survival of the Urotsa cells and primary rat hepatocytes.
- *Statistical methods*

  All data are presented as means±SEM. Data set were subjected to ANOVA (one-way or two-way) followed by Dunnetts's post-hoc analysis or unpaired t-test (GraphPad Prism, GraphPad Software, Inc.,). $p \leq 0.05$ was accepted as the level of significance.

Results and discussion

When incubated with freshly isolated rat hepatocytes for 1 h, BMP did not increase the percentage of DNA in the comet tail (T%DNA) at any of the tested concentrations compared to the vehicle control. The positive control, H_2O_2 caused DNA strand breaks in hepatocytes as the T% DNA was significantly increased at the concentrations of 100 and 50 μM. In URotsa cells, incubation with BMP (10-100 μM) for 1 hr resulted in a concentration dependent increase in the T% DNA. URotsa cells were also more sensitive to H_2O_2 than hepatocytes when the T%DNA was compared at 50 μM, a concentration used for both cell types. Furthermore, BMP binding to hepatocytes DNA decreased significantly with time when compared to BMP binding to the DNA of URotsa cells. In addition, rat hepatocytes converted BMP to BMP-glucuronide while this activity was lacking in URotsa cells. Incubation of rat hepatocytes for 1 h with BMP (100 and 500 μM9 significantly decreased the intracellular GSH concentration. This depletion of GSH was not observed in URotsa cells incubated with the same concentrations of BMP. Potassium bromate ($KBrO_3$, 2mM), the positive control used in the assay, significantly depleted intracellular GSH in both celltypes. Administration of NAC (2mM) as an exogenous source of thiols and a GSH precursor significantly increased the intracellular GSH in URotsa cells.

The present in vitro study investigated the susceptibility of target (UROtsa cells) and non-target cells (primary rat hepatocytes) to BMP-induced genotoxicity. In contrast to hepatocytes, BMP exhibited greater genotoxic potential in UROtsa cells as evidenced by the concentration dependent increase in DNA strand breaks and DNA binding. Total content of intracellular GSH quantified in UROtsa cells (2.7. +/-. 1.0. nmol/mg protein) was 4 fold lower than that in hepatocytes (10.7. +/-. 0.3. nmol/mg protein). HPLC analysis indicated BMP was not metabolized and/or consumed in UROtsa cells at any of the concentrations tested (10-250. muM) but was extensively converted to a mono-glucuronide in hepatocytes. These results demonstrate that a target cell line such as UROtsa cells are more susceptible to BMP-induced DNA damage when compared to non-target cells. This increased susceptibility may relate to the deficiency of antioxidant and/or metabolic capabilities in UROtsa cells. One explanation for this difference is that rat hepatocytes can convert BMP to a less DNA reactive metabolite, an ether glucuronide, while URotsa cells lack the ability to bio-transform BMP. These data support a detoxification role of glucuronidation in BMP associated genotoxicity.

2.1.2 Animal data

2.1.2.1 NTP (1996) Mouse bone marrow micronucleus studies

Study reference:

NTP (1996). NTP Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138(R)) (CAS No. 3296-90-0) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series, U.S. Department of health and human services**:** 1-377.

Detailed study summary and results:

Test type: Mouse bone marrow micronucleus studies

The study is GLP compliant

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes,
- *Degree of purity, Impurities:* 79% pure, impurities identified, *see figure 3 below.*

  The impurities are higher than in most other studies. A (Q)SAR and read-across check on the main impurities imply that these impurities have similar toxicological properties from BMP, or do not contribute to the toxicity (data not shown).
- *Batch number:* 840429-162

Test animals

- *Species/strain/sex:* Male B6C3F1 mice
- *No. of animals per sex per dose:* 5 animals per dose group
- *Age and weight at the study initiation:* Not specified

Administration/exposure

Doses/concentration levels, vehicle, rational for dose selection: 0, 100, 200, 300, 400 mg/kg

- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Corn oil
- *Details on test system and conditions, and details on route of administration, exposure:* Gavage
- *Actual doses given above*

- *Duration of study, frequency of treatment, sampling times and number of samples:* 3 dose gavage protocol, with BMP administered at 24 h intervals followed by bone marrow sampling 24 h after the third dosing
- *Control groups and treatment:* Positive control mice received injections of 12.5 mg dimethylbenzathracene per kg body weight. Negative Solvent control animals were administered corn oil alone
- *Positive and negative (vehicle/solvent) control data*
- *Methods of slide preparation*
- *Criteria for scoring and number of cells analysed per animal:* In the gavage study, 2000 polychromatic erythrocytes (PCEs)were scored for frequency of micronucleated cells in each of the 5 animals per dose group
- *Statistical methods:* For the three-treatment gavage study, the frequency of micronucleated cells among polychromatic erythrocytes (PCEs) was analysed by a statistical software package which employed a one-tailed trend test across dose groups and a t-test for pairwise comparisons of each dose group to the concurrent control

PROCUREMENT AND CHARACTERIZATION OF 2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL

2,2-Bis(bromomethyl)-1,3-propanediol was obtained from Dow Chemical Company (Rolling Meadows, IL) in one lot (840429-162) which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Reports on analyses performed in support of the 2,2-bis(bromomethyl)-1,3-propanediol studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a fine white powder, was identified as 2,2-bis(bromomethyl)-1,3-propanediol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity was determined by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, and gas chromatography. Elemental analyses for carbon, hydrogen, and bromine were in agreement with the theoretical values for 2,2-bis(bromomethyl)-1,3-propanediol. Karl Fischer water analysis indicated 0.3% ± 0.1% water. Thin-layer chromatography by two systems indicated a major spot and one impurity. Gas chromatography using one system indicated one major peak and three impurities, and a second system indicated a major peak and four impurities. In both cases, the total impurity peak area was less than 3%. High-performance liquid chromatography analyses detected multiple impurities with five impurity peaks having areas of 1% or greater relative to the major peak area. The overall impurity peak area was 21.2%. Four impurities were isolated for identification by mass spectrometry. Two impurities, 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane (6.6%) and 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane (6.9%), were identified. One impurity (1%) was tentatively identified as a dimer of the parent chemical. Another impurity peak (2.8%) consisted of multiple components, including a structural isomer and a dimer of the major component. A quantitative analysis for pentaerythritol, a reactant in the synthesis of 2,2-bis(bromomethyl)-1,3-propanediol, was also conducted. Using a reference standard, 0.2% pentaerythritol was found. The overall purity for lot 840429-162 was determined to be approximately 79%.

Stability studies, performed by the analytical chemistry laboratory using gas chromatography, found that 2,2-bis(bromomethyl)-1,3-propanediol was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed containers protected from light. Stability was monitored monthly during the 13-week and 2-year studies using gas chromatography. No degradation of bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared weekly by mixing 2,2-bis(bromomethyl)-1,3-propanediol with feed (Table I1). Homogeneity and stability studies were performed by the analytical chemistry laboratory using gas chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 3 weeks when stored in the dark at −20° C. During the 13-week and 2-year studies the dose formulations were stored in the dark at −20° C for no more than 3 weeks.

Periodic analyses of the dose formulations of 2,2-bis(bromomethyl)-1,3-propanediol were conducted at the study laboratory and analytical chemistry laboratory using gas chromatography. During the 13-week studies, dose formulations were analyzed at the beginning, midpoint, and end of the studies (Table I2). During the 2-year studies, dose formulations were analyzed at least every 10 weeks (Table I3). Of the dose formulations analyzed, 92% (119/130) were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table I4).

Figure 3: Purity, impurities, stability of the NTP (1996) batch of BMP

Results and discussion

In the first two mouse bone marrow micronucleus tests performed, inconsistent results were obtained between two trials which used the same dose range of 100-400 mg/kg BMP, administered by gavage three times at 24 h interval. Results from the first trial were negative while the second trial gave a clear dose-related increase in micronucleated PCEs. The results were therefore concluded as equivocal.

See also the summary above, figure 2 (Summary on genotoxicity from the NTP (1996) report).

2.1.2.2 NTP (1996) Mouse bone marrow micronucleus studies

Study reference:

NTP (1996). NTP Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138(R)) (CAS No. 3296-90-0) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series, U.S. Department of health and human services**:** 1-377.

Detailed study summary and results:

Test type: Mouse bone marrow micronucleus studies

Guideline:The study followed GLP

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes
- *Degree of purity, Impurities:* 79% pure, impurities identified ***(Error! Reference source not found.)***

  The impurities are higher than in most other studies. A (Q)SAR and read-across check on the main impurities imply that these impurities have similar toxicological properties from BMP, or do not contribute to the toxicity (data not shown).
- *Batch number:* 840429-162

Test animals

- *Species/strain/sex:* Male and female B6C3F1 mice
- *No. of animals per sex per dose:* 3 or 4 animals per dose group
- *Age and weight at the study initiation:* Not specified

Administration/exposure

Doses/concentration levels, vehicle, rational for dose selection: 1-dose intraperitoneal injection.

Dosage: 0, 100, 200, 400 mg/kg

- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Corn oil
- Details on test system and conditions, and details on route of administration, exposure: Intraperitoneal injection
- *Duration of study, frequency of treatment, sampling times and number of samples:* 48 h, samples were collected from all the animals that were included in the study

- *Control groups and treatment:* Positive control mice received injections of 200 mg Urethane per kg body weight. Negative Solvent control animals were administered corn oil alone
- *Positive and negative (vehicle/solvent) control data*
- *Methods of slide preparation*
- *Criteria for scoring and number of cells analysed per animal*: In the intraperitoneal injection study, 1000 polychromatic erythrocytes (PCEs) were scored for frequency of micronucleated cells in each of the 3 or 4 animals per dose group
- *Statistical methods:* Data from the single injection micronucleus test were analysed by the Cochran-Armitage trend test and pairwise comparison of dose groups to the corresponding negative controls were made using t-test

Results and discussion

In an attempt to clarify the results obtained in first bone marrow micronucleus test, a second investigation was performed using both male and female mice. BMP was administered as a single intraperitoneal injection (150-600 mg/kg) and bone marrow samples were taken 48 h after dosing. The results showed that BMP induced micronuclei in bone marrow of cells of female mice.

In conclusion, BMP was genotoxic in vivo, inducing micronuclei in erythrocytes of male and female mice.

See also the summary above, figure 2 (Summary on genotoxicity from the NTP (1996) report).

2.1.2.3 NTP (1996) Mouse feed study

Study reference:

NTP (1996). NTP Toxicology and Carcinogenesis Studies of 2,2-Bis(Bromomethyl)-1,3-Propanediol (FR-1138(R)) (CAS No. 3296-90-0) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series, U.S. Department of health and human Services**:** 1-377.

Detailed study summary and results:

Test type: Mouse peripheral blood micronucleus test

Guideline: The study followed GLP

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes

Test animals

- *Species/strain/sex:* Male and female B6C3F1 mice
- *No. of animals per sex per dose:* 10 animals per dose group
- *Age and weight at the study initiation:* 6-7 weeks old

Administration/exposure

- *Doses/concentration levels, vehicle, rational for dose selection:* 5 doses.

- *Dosage:* 0, 625, 1250, 2500, 5000, 10000 ppm
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Corn oil
- *Details on test system and conditions, and details on route of administration, exposure:* Feed study
- *Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose:* These levels corresponded to approximately 100, 200, 500, 1,300, or 3,000 mg 2,2-bis(bromomethyl)- 1,3-propanediol/kg body weight (males) and 140, 300, 600, 1,200, or 2,900 mg/kg bw (females).
- *Duration of study, frequency of treatment, sampling times and number of samples:* 13 weeks
- *Control groups and treatment:* Negative Solvent control animals were administered corn oil alone. No details on the positive control treatment are described
- *Criteria for scoring and number of cells analysed per animal:* In the feed study, 1000 polychromatic erythrocytes (PCEs)were scored for frequency of micronucleated cells in each of the10 animals per dose group
- *Statistical methods:* Log transformation of the NCE data, testing for normality by Shapiro-Wilk test, and testing for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was analysed by analysis of variance using the SAS GLM procedure. The NCEs data for each dose group were compared with the solvent control using a Student's t-test.

Results and discussion

BMP was shown to be genotoxic in vivo. Significant increases in micronucleated normchromatic erythrocytes were observed in peripheral blood sample obtained from male and female mice exposed to 13 weeks BMP in feed. These increases were observed in the two highest dose groups of males (5000 and 10000 ppm) and the three highest dose groups of females (2500-10000).

See also the summary above, figure 2 (Summary on genotoxicity from the NTP (1996) report).

2.1.2.4 Wada et al. (2014)

Study reference:

Wada K, Yoshida T, Takahashi N, Matsumoto K (2014). Effect of seven chemicals on DNA damage in the rat urinary bladder: A comet assay study. Mutation Research 769 (2014), 1-6

Detailed study summary and results:

Test type:

International Validation of the In Vivo Alkaline Comet Assay for the Detection of Genotoxic Carcinogens Version 14.2, 2014, *http://cometassay.com/JaCVAM.pdf (link checked Oct 2016).*

In vivo mammalian alkaline comet assay (OECD TG 489, 2014)

Test substance

2,2-Bis(bromomethyl)-1,3-propanediol (BMP)

Purity: >98.0% (GC)

CAS Nr.: 3296-90-0

Batch number: no details

Test animals

- *Species/strain/sex:* Male Sprague-Dawley Crl:CD (SD) rats
- *No. of animals per sex per dose: Age and weight at the study initiation: Grouping of animals:*
  No. of animals: 5 animals/group
  Age of animals at onset: 7 weeks
  Average initial weigh of animals at onset: 270/273 for low dose and high group dose , respectively

Administration/exposure

- *Route of administration:* Oral gavage
- *vapour, gas, particulate), other:*
- *duration of test/exposure period: doses/concentration levels, rationale for dose level selection: 24 h*
- *Dosage:* 300 and 600 mg/kg/day, respectively
- *The rationale for dose level selection*: The limit doses of BMP were determined by the DNA damage induced in a dose-range finding study
- *Frequency of treatment: control group and treatment: historical control data: post exposure observation period:* A second dose was given after 21 h interval from the first dose. The animals were sacrificed 3 h after the second dose
- *vehicle:* BMP was dissolved in 0.5% methylcellulose (MC)

Results and discussion

- *mortality and time to death (indicate number died per sex per dose and time to death):* NA
- *clinical signs:* There were no changes in appearance or demeanour of the rats during the course of the study that could be attributed to BMP treatment
- *Body weight gain:* Male rats that were exposed to FR-1138 did not show significant increase in body weights as compared to the controls
- *Food/water consumption. Ophthalmoscopic examination: clinical chemistry: haematology: urinalysis: organ weights: necropsy findings: nature and severity:* There were no consistent deviations in the food consumption for male rats treated with BMP as compared to the controls. There was no abnormal necropsy findings in rats treated BMP

- *Cumulative mortality:* No significant difference between BMP treated rats and controls
- *Palpable mass:* There were no statistical differences between control and treated rats over the course of the 24 h study
- *Haematology:* No significant difference between BMP treated rats and controls
- *Urinalysis:* Not performed
- *Clinical chemistry:* Not performed
- *Organ weights:* Not performed
- *Statistical Analysis:* To evaluate the DNA damage, the mean% tail DNA in each treatment group was compared to the 0.5% Dunnett's test was used to compare multiple groups ($p<0.05$)
- *Comet assay results:* The comet assay results showed that the urinary bladder DNA damage (% tail DNA) was increased in animals treated with BMP at the high dose of 600 mg/kg/day; $p<0.05$. DNA damage (% tail DNA) was not increased in liver cells from animals treated with BMP
- *Necropsy finding:* There were no abnormal necropsy findings in rats treated BMP
- *Histopathological findings:* Histopathology did not show carcinogenic changes in urinary bladder due to BMP exposure in the rats

Conclusion: BMP did not cause DNA damage in the liver; however, it did cause DNA damage in the urinary bladder without cytotoxicity, but only at the high dose.

2.1.3 Other data

2.1.3.1 Treinen et al. (1989)

Study reference:

Treinen, K. A., et al. (1989). "Reproductive Toxicity of 2,2-Bis(bromomethyl)-1,3-propanediol in a Continuous Breeding Protocol in Swiss (CD-1) Mice." Fundam Appl Toxicol **13**(2): 245-255.

Detailed study summary and results:

Test type Continuous Breeding Protocol in Swiss (CD-I) Mice.

Protocol: Reproductive assessment by continuous breeding protocol, part of the NTP. Reliability of study: 1.

The test substance was BMP (CAS No. 3296-90-0) The Dow Chemical Company (Midland, MI, LotNo. MM 05137-636, purity 87.3%. 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%)).

Animals: CD-1 albino outbred (Swiss), Charles River Labs, Kingston NY, SPF quality, 6 w old at arrival.

The effect of BMP on reproduction in Swiss CD-1 mice was evaluated by use of a continuous breeding protocol. Both male and female F0 mice (20 pairs per treatment group, 40 pairs of control animals) were dosed 7 days prior to and during a 98-day cohabitation period.

BMP was administered in the feed at 1000, 2000 and 4000 ppm concentrations. To produce satisfactory homogeneity BMP was premixed with ethyl ether and applied to the feed, followed by evaporation of the solvent from the mixture.

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 Fo animals indicated a specific effect only on female reproductive capacity. At the highest dose, BMP caused a body weight decrease in the Fo animals of both sexes with no effect on relative organ weights. Sperm concentration, motility, morphology, and estrual cyclicity were unaffected by BMP exposure. Histopathology in the Fo 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 to age 74 days and then mated to nonsiblings 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.

2.1.3.2 Bolon et al. (1997)

Study reference:

Bolon B et.al, Differential follicle counts as a screen for chemically induced ovarian toxicity in mice: Results from continuous breeding bioassays, Fundamental and applied toxicology 39, 1-10 (1997)

Detailed study summary and results:

Test type

Ovaries from NTP Reproductive Assessment by Continuous Breeding (RACB) bioassays were used to directly compare differential ovarian follicle counts with reproductive performance for 15 chemicals, among them BMP. Details on the RACB bioassay is given by Treinen et al. (1989) and in the original NTP TR 452 on BMP (National Toxicology Program, 1996) above. The animals received BMP in the feed at 0, 0.1, 0.2, and 0.4% concentration. Ovaries of 10 animals (CD-1 mice) per group from the studies in mice were sectioned. Counts of three follicle classes, namely small, growing, and antral follicles were obtained in every 10^{th} section. Counts were performed by a technician and replicated by a second technician. For BMP, reductions in follicle numbers in Task 4 animals (F_1 offspring of Task 2 parents, i.e. animals exposed to BMP as gametes, during prenatal and postnatal development, and as young adults) were proportional to dose, se figure below. Numbers of small and growing follicles were the best markers for quantifying damage, and these two follicle classes represent the maturation pool of female gametes. Altered follicle counts without apparent reproductive impairment occurred in CD-1 mice at low doses of BMP, indicating this as a more sensitive estimate of chemically induced ovarian toxicity than fertility tests.

TABLE 4
Differential Ovarian Follicle Counts in Control and Chemically Treated Mice

Chemical	RACB task	Chemical dose (%)	Reproductive toxicity[a]	Number of follicles[b]		
				Small	Growing	Antral
BPD	3	0	—	157 ± 108	62 ± 25	7 ± 3
		0.4	Y	32 ± 24[c]	15 ± 12[c]	2 ± 2
	4	0	—	360 ± 145	101 ± 27	10 ± 7
		0.1	N	206 ± 117	80 ± 34	10 ± 5
		0.2	N	114 ± 66[c,d]	56 ± 23[c,d]	7 ± 4
		0.4	Y	29 ± 23[c,d]	27 ± 18[c,d]	5 ± 5

Note. N, no toxicity; Y, toxicity supported by RACB data; Y*, toxicity interpolated from RACB data; NR, not reported.
[a] Denotes altered reproductive outcome based on published RACB test results (Morrissey *et al.*, 1989).
[b] Values represent mean counts (± standard deviation) based on a 10% nonrandom sample (every 10th serial section) from one ovary of each of 10 animals per treatment group (except as noted in Table 2).
[c] Denotes a significant difference from controls by the Mann–Whitney U test, $p \leq 0.05$.
[d] Denotes a significance between dose groups by the Kruskal–Wallis nonparametric ANOVA, $p \leq 0.05$.

Figure 4: Excerpt from Table 4 in Bolon et al., 1997

2.2 Carcinogenicity

2.2.1 Animal data

2.2.1.1 Dunnick & al (1997)

Study reference:

Dunnick J. K., Heath J.E., Farnell D. R, Prejean J. D., Haseman J. K., Elwell M. R. (1997). Carcinogenic activity of the flame retardant, 2,2-bis(bromomethyl)-1,3-propanediol in rodents, and comparison with the carcinogenicity of other NTP brominated chemicals. Toxicol Pathol. 1997 Nov-Dec;25(6):541-8.

https://echa.europa.eu/registration-dossier/-/registered-dossier/7873/7/8

Exp Key Carcinogenicity 001

Detailed study summary and results:

Test type: Feed study (OECD TG, 2014)

Test substance

2,2-Bis(bromomethyl)-1,3-propanediol (BMP), CAS number: 3296-90-0

- *Degree of purity, Impurities:* 79% pure, impurities identified ***(Error! Reference source not found.)***

  Impurities: 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane

  6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane

  0.2% pentaerythritol

  7.7% dimers and structural isomers

  (see also **Error! Reference source not found.**)
- *Batch number* 840429-162

Test animals

- *Species/strain/sex:* Male and females Fischer 344 (F344)/N rats and B6C3F1 mice
- *No. of animals per sex per dose:* 60 animals/sex/species/dose
- *Age and weight at the study initiation:* 6 weeks old animals were used at onset . No information is provided about the body weight at onset

Administration/exposure

- *Route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other: Feed ad libitum*
- *duration of test/exposure period: 2 years*
- *doses/concentration levels, rationale for dose level selection:*

  BMP was administered in the diet for 2yr at 0, 2,2000, 5000, or 10,000 ppm to F344 rats and 0, 312, 625, or 1250 ppm to B6C3F1 mice for 104 weeks. The maximum dose selected for rats in the 2-yr study was 10,000 ppm and was based primarily on decreased body weight gain and hyperplasia of the urinary bladder epithelium (males only) at 20,000 ppm BMP dose initial validity test. The 20,000 ppm dose was selected for the recovery study to determine the potential for regression or progression of the transitional cell hyperplasia in the urinary bladder and papillary degeneration of the kidney, which occurred at this dose in most male rats after 13 weeks exposure. The high dose of 1250 ppm selected for mice in the 2-yr study was based on the presence of papillary necrosis, fibrosis, and renal tubule regeneration in the kidney at 2500 ppm after 13 weeks of exposure.
- *Rationale for dose level selection:* The NTP report states: In a study by Keyes et al., (1979, see study 3.9.1.2 in this appendix), carcinogenic effect was observed. However, degenerative changes in the liver and lens of the eye were attributed to chemical exposure. The article did not provide details on the preparation or stability of the chemical in the feed. No dose-related effects on the feed consumption, weight gain, clinical signs, or mortality were observed, suggesting that the animals may have been able to tolerate higher doses. Hence, the doses chosen in the NTP study were higher than in the Keyes study.
- *frequency of treatment:* Feeding ad libitum
- *control group and treatment:* Rat and mice fed no BMP
- *historical control data:* No details presented in the report

- *post exposure observation period:* A stop exposure group was also conducted in male rats; 60 animals received 20,000 ppm BMP for three months and then were continued on normal diet
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water:* NIH 07 feed
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* Formulated diets were prepared by mixing appropriate amounts of BMP with NIH 07 feed. FR- 1138 was chosen for the study because it was used by Keyes & al., and because it was the currently used mixture of BMP.
- *substance concentration (ppm) to the actual dose:*

  Ppm to mg/kg bw conversion:

  F344 rats: 0, 2500, 5000, 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 was included; 60 animals received 20,000 mg/kg (approx. 800 mg/kg bw) BMP for three months and then were continued on normal diet

  B6C3F1 mice: 0, 312, 625, or 1250 ppm (mg/kg) in feed equivalent to

  Males: 0, 35, 70, 140 mg/kg bw

  Females: 0, 40, 80, 170 mg/kg bw
- *satellite groups and reasons they were added:* A stop exposure group was also conducted in male rats; 60 animals received 20,000 ppm BMP for three months and then were continued on normal diet

Results and discussion

- *mortality and time to death (indicate number died per sex per dose and time to death):*

  There was decreased survival in rats at 5,000 and 10,000 ppm, in male from the stop-exposure group (20,000 ppm) and in mice at 1,250 ppm. This was primarily a result of treatment-related neoplasms in animals from these dose groups.
- *Clinical signs:* An exposure-related carcinogenic effect was observed at 17 sites in male rats, and 4-6 sites in female rats, male and female mice, respectively.
- *body weight gain:* Mean body weights of male rats at 10,000 ppm and 20,000 ppm and female rats 10,000 ppm were 5-15% lower than controls throughout most of the study; mean body weights of exposed mice were similar to controls throughout most of the study.
- *Food/water consumption. Ad libitum*. Food consumption by exposed rats was generally similar to that by controls throughout the study. In 20,000 ppm stop-exposure males, the feed consumption was lower than that by controls.
- *ophthalmoscopic examination*: Not described
- *clinical chemistry:* Not described in the paper, but in the NTP report
- *haematology:* Not described in the paper, but in the NTP report

- *urinalysis:* Not described in the paper, but in the NTP report
- *organ weights:* Not described in the paper, but in the NTP report
- *necropsy findings: nature and severity:* A complete gross necropsy was performed on all animals from all groups that died or were sacrificed during and at the end of the experiment. All major organs and tissues (> 40) and grossly observed lesions and masses were trimmed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. The results are presented under histopathological finding (nature and severity below).
- *Statistical Analysis:* Differences in survival of animals were analysed by life table method. For the analysis of tumor incidence data, survival adjusted procedures were used to assess dose-response trends and to make pairwise comparison between dosed groups and controls. Fischer exact tests and Cochran-Armitage trend tests were also utilized to analyse tumor incidence data. P value <0.05 was considered significant
- *histopathological findings, nature and severity:*

Table 3: Summary table of carcinogenic activity in 2-yr study of BMP

Site	*Rats*		*Mice*	
	Male	*Female*	*Male*	*Female*
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	+			
Hematopoietic system	+			
Pancreas	+/-			
Harderian gland			+	+
Circulatory system				+/-

+ = some or clear evidence of carcinogenic activity; +/- = equivocal evidence of carcinogenic activity

Table 4: Overview of treatment related non-neoplastic and neoplastic lesions in the male rat dosed for 3 mo (stop exposure) or 2-yr with BMP.[a]

Site	*Dose (ppm)*					*Dose response*
	0	*2.500*	*5.000*	*10.000*	*20.000 (stop exposure*	
Skin	*4*	*7*	*14***	*24***	*21***	
Subcutaneous tissue	*2*	*9**	*13***	*18***	*10***	
Zymbal gland	*2*	*1*	*4*	*5*	*17***	*Yes*
Oral cavity	*0*	*4**	*9***	*10***	*14***	
Esophagus	*0*	*0*	*1*	*6**	*0*	
Forestomach	*0*	*0*	*0*	*1*	*5**	*Yes*
Small intestine	*0*	*0*	*0*	*2*	*5**	*Yes*
Large intestine	*0*	*0*	*3**	*4**	*12***	*Yes*
Peritoneum	*0*	*3*	*8***	*9***	*26***	*Yes*
Kidney	*10*	*25***	*55***	*88**	*43***	
Urinary bladder	*0*	*0*	*2*	*6***	*12***	*Yes*
Lung	*4*	*5*	*8**	*11**	*24***	*Yes*
Thyroid	*1*	*2*	*8**	*8**	*15***	*Yes*
Seminal vesicle	*1*	*6*	*4*	*16***	*33***	*Yes*
Hematopoietic system	*27*	*29*	*40***	*34***	*25***	
Mammary gland	*0*	*4*	*7***	*7***	*5***	
Pancreas	*4*	*11**	*16***	*17***	*30***	*Yes*

[a]*Number of animals with lesions in group (51-60 animals examined per group); *p<0.05 and **p<0.01 vs controls*

Table 5: Overview of treatment related non-neoplastic and neoplastic lesions in the female rat treated with BMP for 2-yr. [a]

Site	Dose (ppm)				Dose response
	0	2.500	5.000	10.000	
Mammary gland	27	47**	47**	47**	
Oral cavity (all tumors)	2	3	5	6	

Esophagus	0	0	0	10**	
Kidney	0	5	5	28**	
Thyroid	0	0	2	4**	Yes

[a]Number of animals with lesions in group (50-53 animals examined per group); *p<0.05 and **p<0.01 vs controls

Table 6: Overview of treatment related nonneoplastic and neoplastic lesions in male mice treated with BMP for 2-yr. [a]

Site	Dose (ppm)				Dose response
	0	2.500	5.000	10.000	
Hardenian gland	4	7	16**	22**	Yes
Lung	15	11	16	25*	Yes
Forestomach	0	3	3	4*	
Kidney	0	0	3	2	

[a]Number of animals with lesions in group (50-53 animals examined per grpoup); *p<0.05 and **p<0.01 vs controls

Table 7: Overview of treatment related nonneoplastic and neoplastic lesions in female mice treated with BMP for 2-yr. [a]

Site	Dose (ppm)				Dose response
	0	2.500	5.000	10.000	
Hardenian gland	3	12**	13**	19**	Yes
Lung	6	8	23**	34**	Yes
Skin, subcutaneous	0	1	4	12**	Yes
Forestomach	0	1	5*	3*	
Mammary gland	0	0	1	3	
Circulatory system	1	2	0	5*	

[a]Number of animals with lesions in group (50-53 animals examined per grpoup); *p<0.05 and **p<0.01 vs controls

Treatment related carcinogenetic activities were observed in seventeen sites in male rats and 4 sites in female rats (Table 1). Most activities in the kidney and pancreas of the male rats were predominantly hyperplastic. Consequently, the carcinogenetic status of these organs was defined as equivocal. Otherwise, the other fifteen organs in the male rats displayed different types of adenomas, predominantly, on exposure to BMP. In male mice treated with BMP, four carcinogenetic activity sites were observed while three organs exhibited carcinogenic activities in female mice (Table 1). In the female mice mammary gland and circulatory system, carcinogenic activities were observed at the highest BMP dose; hence, the effect of BMP treatment on these organs were not dose-dependent.

BMP dose-response observations in both the satellite (3 mo stop exposure) and the main study groups showed a treatment-related non-neoplastic and neoplastic lesions in both rats and mice, irrespective of sex

(Tables 2, 3, 4, 5). A common motive in the dose-respond carcinogenetic activity in both the rats and mice, irrespective of sex, was observed in the lungs. There was a treatment-related increase in neoplasm of the upper gastrointestinal tract (oral cavity, tongue and esophagus in male and female rats). In addition, there were treatment-related neoplasms in the small and large intestine in male rats.

In conclusion, BMP was carcinogenic in rodents. Sites for cariogenic activity for BMP include oral cavity, intestine and kidney, among others.

2.2.1.2 Keyes & al (1980)

Study reference:

Keyes, D.G., Kociba, R.J., Schwetz, R.W., Wade, C.E., Dittenber, D.A., Quinn, T., -Gorzinski, S.J., Hermann, E.A., Momany, J.J., and Schwetz, B.A. (1979). Results of a two-year toxicity and oncongenic (sic) study of rats ingesting diets containing dibromoneopentyl glycol (FR- 1138). J. Combustion Toxicol. 7, 77-98.

Detailed study summary and results:

Test type: Feed study (OECD TG 453, 2014)

Test substance

FR-1138

A mixture of the following:

- 80% dibromeopentyl glycol (BMPD)
- 8% tribromeopentyl alcohol
- 6% monobromeopentyl tricol
- 3% other impurities

Test animals

- *Species/strain/sex:* Male and female Sprague-Dawley rats
- *No. of animals per sex per dose: Age and weight at the study initiation: Grouping of animals:*

  1. 49-50 animals/sex

  2. 5 animals/sex/group for one year interim kill

  3. 10 animals/sex/group for tissue analysis

  Age of animals at onset: 7-8 weeks

Administration/exposure

- *Route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other:* Feed
- *duration of test/exposure period: doses/concentration levels, rationale for dose level selection: 2-yr*

  Dosage: 0, 5, 100 mg/kg/day

  The rationale for dose level selection is not presented in the report
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water: test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation: actual doses (mg/kg bw/day) and conversion factor from*

diet/drinking water test: The test diet were prepared by mixing FR-1138 with ground Purina Laboratory Chow to make a 5% premix. The concentration of the test material was adjusted on a weekly basis for the first three months, and quarterly thereafter to maintain the designated dose levels on mg/kg of body weight/day basis according to the mean food consumption and body weight data. Control rats were supplied with untreated ground laboratory chow.

Results and discussion

Pathology:

A gross necropsy was performed on all animals from all groups that died or were sacrificed during and at the end of the experiment.

All major organs and tissues and grossly observed lesions and masses were trimmed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analysis:

Data on mortality, palpable masses, gross pathology, histopathology and tumor incidences of the rats of the 2-years study were analysed using Fischer's Exact Probability Test, $P<0.05$, one sided test. For gross pathology observations, statistical evaluation of the cumulative data for the entire study compared the data of each of the treatment groups against the data of the control group of that sex

- *mortality and time to death (indicate number died per sex per dose and time to death):* Males showed no changes in mortality. Females given low dose showed no changes in mortality. Females given high dose had a statistical increased mortality rate at months 16-17, considered of questionable toxicological significance.
- *clinical signs:* There were changes in appearance or demeanour of the rats during the course of the study that could attributed to FR-1138 in the diet
- *Body weight gain:* Both male and female rats that were exposed to FR-1138 did not show significant increase in body weights as compared to the controls.
- *Food/water consumption. ophthalmoscopic examination: clinical chemistry: haematology: urinalysis: organ weights: necropsy findings: nature and severity:* There were no consistent deviations in the food consumption for male and female rats treated with FR-1138 as compared to the controls
- *Cumulative mortality:* Male rats given 5 or 100 mg/kg/day FR-1138, showed no difference in mortality when compared to the control group. Female rats receiving 100 mg/kg/day had a statistical increased mortality rates for months 16-17. Mortality data on female rats given 5 mg/kg/day FR-1138 showed no differences in from control data.
- *Palpable mass:* There were no statistical differences between control and treated rats over the course of the 2-yr study.
- *Haematology:* Repetitive haematological parameters monitored after approximately 90-91, 356-357, 713-714, 725 and 731 days showed no effect which could be considered to be related to treatment.

- *Urinalysis:* Routine analysis of urinary parameters after approximately 90-91, 356-357, 713-714 days revealed no observations for either males or females which were considered to be the result of treatment
- *Clinical chemistry:* Analyses of serum samples from rats killed after 94 days, 1 year, 2 years measuring levels of BUN, SGPT and AP showed no alterations considered to be the results of treatment. Glucose levels of serum samples from rats terminated after 2 years showed no effects which could be considered to be results of treatment

- *Organ weights:* Rats given 100 mg/kg/day which were killed after 90 days showed a statistical increase in the relative kidney weights. Since this effect did not continue to the study termination, it was not considered to be related to treatment. Male rats given 100 mg/kg/day killed after 1 year of treatment showed a statistically significant increase in relative liver weights. This was considered to be a result of treatment. Male rats given 5 mg/kg/day which were killed after one year showed a statistical increase in the relative heart weight; this was not considered of any toxicological significance due to its isolated occurrence.
- *Statistical Analysis:* Haematology, urinary and clinical chemistry parameters, body weights, organ weights and organ/body weight ratio data were statistically analysed by a one-way analysis variance followed by Dunnett's test, $p<0.05$. Food consumption data were analysed using the sequential outlier's test, $p=0.02$ (two sided) to identify outlying points. Data on mortality, palpable masses, gross pathology, histopathology and tumor incidences of the rats 2-year study were analysed using Fisher's exact probability, $p<0.05$, one-sided test. For gross pathology observations, statistical evaluation of the cumulative data for the entire study compared the data of each of the treatment groups against the data of the control group of that sex.

- *Histopathological findings, nature and severity*: After 1 year of treatment, there was no gross observations which were considered related to treatment. Statistical analysis of tumor data and tumor incidences for all categories of tumor types occurring in any of the group given 5 or 100 mg FR-1138/kg/day were comparable to the control group.

Conclusion: BMP had no carcinogenic effects in SD-rats in this study.

2.2.2 Other data (e.g. studies on mechanism of action)

2.2.2.1 Ton et al. (2004)

Study reference:

Ton, T. V. T., et al. (2004). "Predominant K-ras Codon 12 G -> A Transition in Chemically Induced Lung Neoplasms in B6C3F1 Mice." Toxicologic Pathology **32**(1): 16-21.

Detailed study summary and results:

Test type

Feed study (OECD TG 453), the same NTP-experiment as described by Dunnick et al., 1997. (section 3.9.1.1)

Test substance

2,2-Bis(bromomethyl)-1,3-propanediol (BMP)

CAS number: 3296-90-0

Purity: See Dunnick et al., 1997

Test animals *(Refer to Dunnick et al., 1997 for details)*

Species/strain/sex: Male and female B6C3F1 mice

Administration/exposure *(Refer to Dunnick et al., 1997 for details)*

Results and discussion *(Refer to Dunnick et al., 1997 for details)*

- *statistical methods and results (unless already described with specific test results above)*

  Complete necropsy was performed. The lungs were fixed, DNA was isolated from slides and used for manual sequencing of K-ras exons 1 and 2 PCR fragments.

- *Statistical analysis:* The incidence and pattern of K-ras mutations were analysed using one-sided Fisher's exact test. Significance of difference was assumed at $p<0.05$.

- *Other results:* See table below

  In brief, there was significant increase in the frequency of mutations in K-ras gene after exposure to BMP (29/51 of the tissues analysed, $p<0.001$). Codon 12 of the k-ras was the most mutated while few mutations were seen in codon 13. No mutation in K-ras codon 61 were identified in the neoplasms from BMP-exposed or feed control mice. There was no dose-response in the frequency of mutations in K-ras gene (64% (1/11), 62% (16/26) and 43% (6/14) in the lung tumors from 312, 625, and 1250 ppm dose groups, respectively. Specific K-ras mutations were not associated with the morphologic pattern of lung neoplasm.

Table 8: Patterns of K-ras mutations in lung neoplasms from B6C3F1 mice

		Codon 12			
Treatment	Activated K-ras (%)	GTT	GAT	TGT	Codon 13 (GGC) CGC
Historical data[1]	25/84 (30%)	1	9	5	3
Feed control[2]	2/20 (15%)	0	3	0	0
BMPD[2]	29/51 (57%)	7	20	1	1
[1]Spontaneous lung neoplasms of control B6C3F1 mice (Historical data)					
[2]Male and female B6CF1 mice were exposed to 0, 321, 625, 1250 ppm BMPD in feed for 24 mo					

Conclusion: The validity of lung cancers observed in B6C3F1 mice are confirmed by mutation characterization in the target gene K-ras, and the induction of mutations indicate genotoxicity of parent compound or metabolites. A relative high increase in K-ras codon 12 G →transversions (CGT to GTT) was observed in BMP-induced lung neoplasms compared to historical control.

2.2.2.2 Elwell et al. (1989)

Study reference:

Elwell, M. R., et al. (1989). "Kidney and urinary bladder lesions in F344/N rats and B6C3F1 mice after 13 weeks of 2,2-bis(bromomethyl)-1,3-propanediol administration." Fundamental and Applied Toxicology 12(3): 480-490.

Detailed study summary and results:

Test type: *13-week repeated dose study*

- *Gavage administration:* Rats were dosed by oral gavage, 5 ml/kg body wt, to deliver daily doses of 0, 50, 100, 200, 400, and 800 mg/kg. Stock suspensions of BMP in corn oil. Mice were dosed by oral gavage, 10 ml/kg body wt, to deliver daily doses of 0, 25, 50, 100, 200, and 400 mg/kg.
- Feed administration: BMPD 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 4.190 mg/kg bw in females.
- *Rats strain:* Male and female F344/N rats
- *Rats age at onset:* 6-7 weeks
- *Mouse strain:* B6C3F1

  10 animals/sex
- *Mice age*: 6-9 weeks

  10 animals/sex
- *Dosage:* Animals were dosed 5 days per week for 13 weeks, and necropsied within one day of the last dose. Animals in the feed study received chemical ad libitum; feed consumption was measured weekly.

Results and discussion

- *Statistical analysis:* Group means and standard deviations were calculated for terminal body weights and clinical chemistry parameters. The significance of differences between the means of treated and control groups was assessed using multiple comparison procedures, $p < 0.05$ was taken as the threshold of statistical significance.
- *Pathology:* Complete gross necropsies were performed and the tissues evaluated histopathologically.

Results: Hyperplasia of the transitional cell epithelium occurred in the urinary bladder of male rats and of mice of both sexes in the feed and gavage studies at the higher doses.