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 [14CBMP] after intravenous*	*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* *Doses provided 25 to 200 μCi/kg*	(Hoehle *et al.*, 2009)

Method	Results	Remarks	Reference
	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.	*[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 intravenous blood kinetics [14C]BMP (10 or 15 mg/kg; 50*	

Method	*Results*	*Remarks*	*Reference*
		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 characterized total blood from rats after intravenous (2 F-344 rats; 10 mg/kg; 200μCi; I ml) or oral (4 F-*	

Method	***Results***	***Remarks***	***Reference***
		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 microsomes converted TFMU into TFMU glucuronide; indicating that human micrososmes*	*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 by tryptan blue exclusion*	(Rad *et al.*, 2010)

Method	Results	Remarks	Reference
	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).*	*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 hepatocyte incubations, respectively, were collected at various times and analysed by*	

Method	*Results*	*Remarks*	*Reference*
		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 ($t1/2\alpha$ = 3.4 min), a significant slower elimination ($t1/2\beta$ = 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

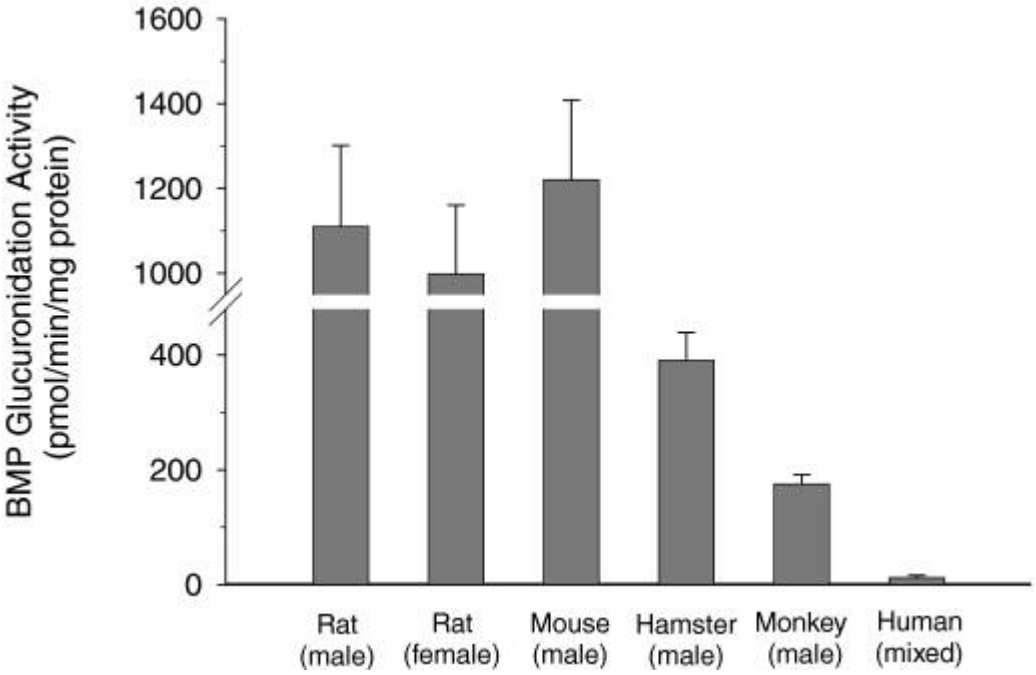

Figure 1: Activities of hepatic microsomes from male and female F-344 rats, male B6C3F1 mice, male Golden Syrian hamsters, male rhesus monkeys, and humans (mixed gender) for the glucuronidation of BMP. Rates of glucuronidation were determined at 50 µM BMP and are expressed as picomoles per minute per 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.