Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol

EC Number: 253-057-0
CAS Number: 36483-57-5; 1522-92-5

CLH-O-0000006818-61-01/F

Adopted

11 June 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: 2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol
EC number: 253-057-0
CAS number: 36483-57-5; 1522-92-5
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Netherlands	ICL Europe Cooperative U.A.	Company-Manufacturer	1
Comment received				
The brominated flame retardant, 2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol (TBNPA, TR-513, Trinol, CAS #36483-57-5) was assessed by the Norwegian Environment Agency for germ cell mutagenicity, carcinogenicity, and reproduction. Based on a read-across approach with the structural analog 2,2- bis(bromomethyl)propane- 1,3-diol (BMP, FR-522, Dinol, CAS #3296-90-0) the CLH classification was proposed for mutagenicity of Muta 1B, H340 and for carcinogenicity of Carc 1B, H350. Classifications for developmental and reproductive toxicity were not determined as the results from the prenatal developmental toxicity study did not warrant classification. ICL believes that the proposed classification for mutagenicity and carcinogenicity is unjustifiably severe and the reproduction classification is unclear. Accordingly, ICL is providing comments on the proposed mutagenicity, carcinogenicity and reproduction classifications as proposed in the 14 June 2019 CLH report. As indicated below as well, ICL request a postponement of the classification proposal until an ongoing relevant study has been completed, which take another 7 month. The attachment contains more details on the Mutagenicity and Carcinogenicity as well as comparison to the CLP criteria. ECHA note – An attachment was submitted with the comment above. Refer to public attachment TBNPA comments to CLH 23092019.pdf				
Dossier Submitter's Response				
Thank you for your detailed comments. Regarding the ongoing study we note, based on the ECHA decision, that you have the requirement to submit the study and update the registration dossier by 25 March 2020. It is up to ECHA to decide whether to postpone the discussion until the study is available, but we agree that it seems reasonable to wait for this as it improves the database.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

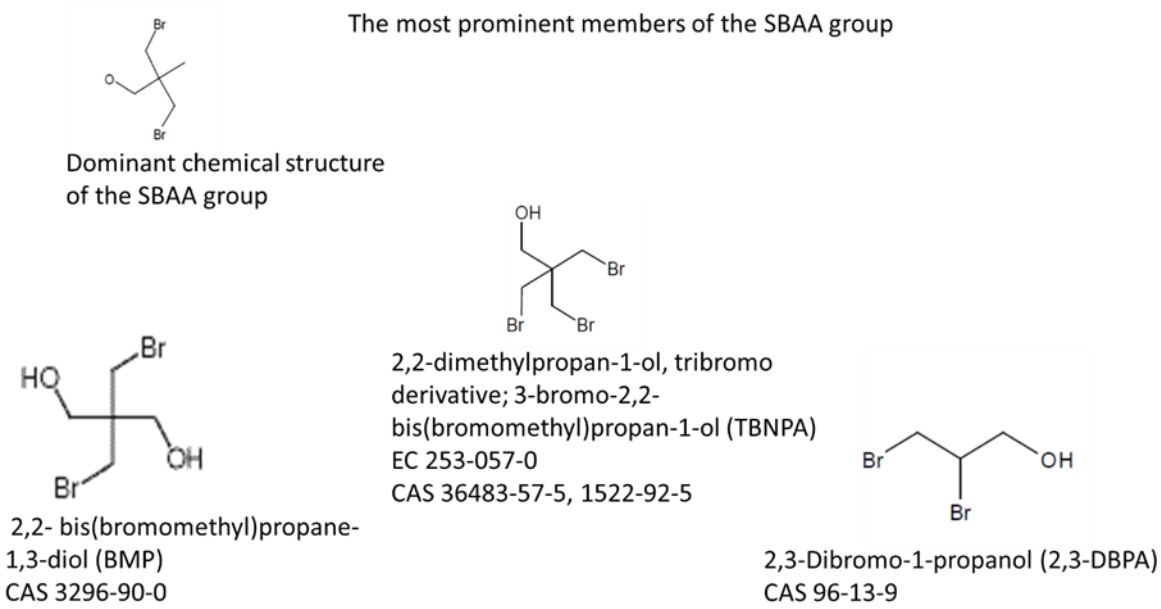
Regarding your attachment, we have responded to this in the specific comments below.

RAC's response

The DS proposed read-across to BMP. RAC has also identified another similar substance, 2,3-DBPA, which has a harmonised classification and labelling (Annex VI, Index number 602-088-00-1, CLP 00) and could also be used for read-across for the classification of TBNPA.

2,3-DBPA is mentioned in the CLH dossier as member of the Small Brominated linear and branched Alkyl Alcohols (SBAA) group described by the Danish Environment Protection Agency (DEPA) in its respective report entitled "Category approach for selected brominated flame retardants - preliminary structural grouping of brominated flame retardants" (Wedebye *et al.*, 2016). This SBAA group was originally predicted by a number of (Q)SAR models including the OECD QSAR Toolbox. The members of the SBAA group (61 identified in the DEPA report) had *a priori* very similar chemical structures with 3-5 carbons, 2-3 bromine atoms and 1-2 alcohol groups (see the following Figure).

Figure*:* Chemical structures and identifiers of TBNPA, BMP and 2,3-DBPA



Regarding chemical similarity between TBNPA and BMP the following can be noted:

- ✓ In TBNPA, one OH group is replaced by one Br, making TBNPA less symmetric, more polarized and more reactive compared to BMP
- ✓ In both substances, all the Br and OH groups are attached to primary carbons (labile C-Br bond, reactive hydroxyl groups)
- ✓ Both substances share a common 5 carbon backbone

Regarding chemical reactivity of TBNPA and BMP the following can be noted:

- ➢ Both substances share similar electrophilic properties of the base molecule
- ➢ For both substances, nucleophilic substitution of the Br (more labile) can take place and/or of the OH group, when enzymatically activated
- ➢ For both substance, radical activation is possible, which constitutes also an alert for a genotoxic mechanism
- ➢ For both substances, the aliphatic halogen is a structural alert both for carcinogenicity and mutagenicity

Regarding chemical similarity between TBNPA and 2,3-DBPA the following can be noted:

- ✓ Br and OH groups attached to primary carbons
- ✓ Both substances share a common 3-carbon backbone

Nevertheless, the chemical structure of 2,3-DBPA, which comprises 2 carbon atoms less than both TBNPA and BMP, has a Br group on a secondary carbon, vicinal both to a primary carbon Br and primary carbon OH group, which renders 2,3-DBPA more reactive compared to both TBNPA and BMP and through different mechanisms, which probably do not operate in the other 2 SBAAs. More specifically, dehydrohalogenation of 2,3-DBPA has been experimentally proven by the detection of 2-bromoacrylic acid as a metabolite. In addition, oxidation to form epoxides, which can enter different metabolic pathways, can also take place. Despite this, all the mechanisms and the structural alerts described above for TBNPA and BMP cannot be excluded for 2,3-DBPA

In the DEPA report, it is explained that all members of the SBAA group have specific structural alerts for mutagenicity and carcinogenicity, for example the "aliphatic halogen" (alert for *in vitro* and *in vivo* mutagenicity and carcinogenicity in the OECD QSAR Toolbox). This alert identified 34% false positives among the mutagenicity training set chemicals (Kazius *et al.*, 2005). According to Benigni *et al.* (2008 and 2010), this alert has a positive predictivity for carcinogenicity of 74%. Nevertheless, as there are multiple chemical reactions possible in a biological system, it does not seem that there is one single mechanistic interpretation to explain this alert in relation to mutagenicity and cancer. Some alerts were identified in all the SBAA group members and/or their metabolites pointing to possible common mechanism(s) of action (e.g. metabolic activation to reactive carbonyl compounds and aldehyde Schiff-base formation of DNA adducts and cross-links). In addition, the same nucleophilic substitution (S_N2) reaction mechanism, which has been proposed as the primary method of DNA alkylation, is expected to be shared due to the presence of the bromide group (Sobol *et al*., 2007). In the DEPA report, BMP and TBNPA were found to belong to the same (Q)SAR-based clusters identified for genotoxicity and carcinogenicity, 2,3-DBPA (which has harmonised classification as Carc. 1B and Repr. 2; H361f) did not result in the same clusters for genotoxicity and carcinogenicity as BMP and TBNPA. For reproductive toxicity the three substances are in separate clusters (positive predicted indications in several reproductive toxicity models). All three substances have similar profiles for endocrine activity and skin sensitization (positive predicted indications for airway allergy).

The physicochemical and structural properties for TBNPA, BMP and 2,3-DBPA that are of interest in the present ODD are summarised in the following table. The physicochemical properties between the 3 SBAAs present similarities and differences, with some properties of 2,3-DBPA lying between the values reported for TBNPA and BMP (relative density, LogPow, ALogP). TBNPA is considerably less soluble compared to BMP and 2,3-DBPA. Nevertheless, availability of TBNPA in biological fluids, where the temperature is higher (around 36°C compared to 20°C), is expected to be enough for the substance to exert similar toxicological effects as BMP and 2,3-DBPA. These toxicological effects are due to the similar chemical structure/functional groups and comparable physico-chemical properties of these three SBAAs.

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Table: Summary of physico-chemical properties and structural features for TBNPA, BMP and 2,3-DBPA

Property	**TBNPA** [1,2]	**BMP** [1,2,3]	**2,3-DBPA** [2,4]
Physical state at 20°C and 101.3 kPa	Solid, white to off-white flakes	Off white crystalline powder, odourless	Clear colorless to slightly yellow viscous liquid
Melting point	Melting / freezing point at 101 kPa: 68.96 °C	Melting / freezing point at 101 KPa: 109 °C	-
Flash point	-	-	> 235 °F (113 °C)
Boiling point	-	270 °C at 101 KPa	426 °F (219 °C) at 760 mm Hg (101 KPa)
Relative density	2.286 at 20°C	1.2 at 20°C	2.120 at 20 °C/4 °C
Vapour pressure	0±0.21 kPa at 25°C	0.85 KPa at 25 °C	1 mm Hg (0.13 Kpa) at 134.6 °F (57 °C)
Polar surface area	20.23	40.46	20.23
Water solubility	1.93 g/L at 20 °C	19.4 g/L at 20 °C	50 to 100 g/L at 68° F (at 20 °C)
Partition coefficient n-octanol/water Log Kow (Log Pow)	2.6 at 22.5°C (2.47)	0.85 (1.06)	- (1.13)
ALogP[5]	2.15	0.75	1.14
Hydrogen bond acceptors	1	2	1
Hydrogen bond donors	1	2	1
Rotable bonds	4	4	2
Lipinski score[6]	0	0	0
Molecular weight	324.8	261.9	217.9
Parent atom counts	9	9	6

[1] ECHA dissemination site

[2] Danish Environment Protection Agency (DEPA) report entitled "Category approach for selected brominated flame retardants - preliminary structural grouping of brominated flame retardants" (Wedebye et al., 2016)

[3] US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Oct 25, 2010: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

[4] National Toxicology Program, Institute of Environmental Health Sciences, National Institutes of Health (NTP). 1992. National Toxicology Program Chemical Repository Database. Research Triangle Park, North Carolina

[5] Atom based method of measuring distribution coefficients using atomic contributions usually in pharmaceutical industry. The most common elements contained in chemical substances (hydrogen, carbon, oxygen, sulfur, nitrogen, and halogens) are divided into several different atom types depending on the environment of the atom within the molecule. While this method is generally the least accurate, the advantage is that it is the most general, being able to provide at least a rough estimate for a wide variety of molecules

[6] Determines if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans

None of the members are predicted to be persistent or bioconcentrating.

Regarding toxicokinetics and metabolism, there are no data available for TBNPA and the data on BMP are rather limited. More specifically, glucuronidation is the sole established route of metabolism of BMP in liver microsomes or primary liver cells of rodents, Rhesus monkeys and humans. The rate of BMP glucuronidation in rodent cells was 150-fold higher than in human hepatocytes. It is assumed that this is a detoxification route and this is expected to be the same for TBNPA and 2,3-DBPA. In addition, BMP has been detected in the gonads (Hoehle *et al*., 2009). In the testis of rats only 0.01% BMP was recovered after up to 10 days of exposure. No female rats were used in this specific study. There were no other toxicokinetic data examining whether BMP reaches the ovaries of mammals. No data were available on the distribution of the metabolite(s) before and after interal reabsorption, or whether BMP or its glucuronide metabolite is the active compound. On the other hand, similar to many halogenated aliphatic alcohols, in metabolism, 2,3-DBPA is oxidized and dehalogenated. After conjugation to glutathione, the intermediate epoxide is metabolized further to mercapturic acid. As a result of hydrolysis of the epoxide and successive oxidation, bromoacetic acid and oxalic acid may also form. The glutathione conjugate can also be metabolized to a highly reactive episulphonium ion, as a result of which there is the possibility of adduct formation at N-7 of the guanine (NTP, 1993)*. Apart from these experimental findings, all three SBAAs can undergo a variety of different reactions, either through a xenobiotic metabolic pathway (i.e. cytochrome P450 oxidases, UDP-glucuronosyltransferases, glutathione S-transferases) or by interacting with DNA via multiple mode of actions.

Based on the available studies for the three SBAAs, a summary of the toxicological properties identified has been carried out as follows.

The kidney is recognized as the common target organ for all three SBAAs and as the target organ where the most prominent and severe effects were observed in various species (TBNPA – rats, BMP – rats & mice, 2,3-DBPA – male rats). Among the other organs affected by the three SBAAs, the liver was shown to be a common target organ for both TBNPA (rats), with mild effects (reduced SGPT activity, increased liver weight and minimal centrilobular hypetrophy) and for 2,3-DBPA (rats & mice), with the severity of the effects for the latter being equally prominent as those observed in the kidney. Urinary bladder was the common main target organ for TBNPA and BMP. Lung was only targeted by 2,3-DBPA.

With regard to fertility, data exist only for BMP and 2,3-DBPA, and the effects observed reveals some obvious differences as well as some similarities. 2,3-DBPA mainly exerts its action on male reproductive organs, while BMP exerts fertility impairment on F0 and F1 animals, mainly, if not exclusively, to females (litters/pair, fertility index, no live pups/litter). Nevertheless, examination of the available data for BMP, provided by an NTP study using the protocol for Reproductive Assessment by Continuous Breeding (RACB), showed some common effects between BMP and 2,3-DBPA on male fertility parameters: in the F1, absolute testis weight was significantly decreased (16%) at the highest dose, along with significantly decreased epididymal sperm density (14%) after BMP administration. These latter findings are comparable with 2,3-DBPA effects on fertility.

Regarding mutagenicity, a full dataset is available for TBNPA. TBNPA and 2,3-DBPA have the same *in vitro* tests positive (Ames test, Mouse Lymphoma Assay – MLA, Thymidine Kinase – TK mutation test), but 2,3-DBPA is active both with and without metabolic activation, while TBNPA only with metabolic activation. TBNPA and BMP were positive in the same *in vitro* tests (Ames test, chromosome aberration test) both with metabolic activation, but again differences in reactivity are noted. TBNPA is also active without metabolic activation at the highest dose in the chromosome

aberration test, while the Ames test for BMP is reported negative in 10% of metabolic S9 activation mixture and positive only with 30% S9. TBNPA and 2,3-DBPA were negative in the same *in vivo* test (erythrocyte micronucleus test – MN) but with limitations, while BMP was positive in two different *in vivo* erythrocyte MN tests (at higher doses than TBNPA and 2,3-DBPA) as well as in an *in vivo* comet assay in urinary bladder, but was negative in a comet assay in liver cells. It can be concluded that the mechanistic pathways operating for TBNPA and BMP are possibly similar, with TBNPA being slightly more reactive, while 2,3-DBPA shares some common mechanisms but also exhibits extra reactivity compared to the other 2 SBAAs.

Regarding carcinogenicity, there are no data for TBNPA, while the carcinogenic profile of BMP and 2,3-DBPA seems rather similar, with many common tumours in both sexes of rats and mice

RAC has applied the Read-Across Assessment Framework (RAAF) (2017) developed by ECHA for the two possible source substances: BMP and 2,3-DBPA. RAAF renders high to medium confidence for reading across from BMP to TBNPA, while confidence for reading across from 2,3-DBPA to TBNPA is only sufficient (due to the partial similarity of 2,3-DBPA to TBNPA in terms of chemical structure and reactivity, and physicochemical and toxicological properties.

In conclusion, for classification purposes RAC makes use of the available experimental data on TBNPA and, when this is not available, insufficient or inadequate (due to, for example, deficiencies in the testing methods), read-across is carried out from BMP (but not 2,3-DBPA).

For more details on specific hazard classes see in comments #2, #5, #9, #13.

* National Toxicology Program (NTP): Toxicology and carcinogenesis studies of 2,3-DBPA in F344/N rats and B6C3Fi mice (dermal studies). Technical Report Series No. 400. NIH Publication No. 92-2855. National Institute of Environmental Health Sciences, Research Triangle Park, NC (1993).

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Netherlands	ICL Europe Cooperative U.A.	Company-Manufacturer	2

Comment received

ICL believes that the proposed hazard category for carcinogenicity 1B is also too severe. Although BMP and TBNPA were found to belong to the same (Q)SAR-based clusters for genotoxicity and carcinogenicity, the genotoxicity of TBNPA is only observed in vitro and the lack of in vivo gene mutation eliminates genotoxicity as a mode of action for potential carcinogenicity for TBNPA. In addition, the physicochemical properties differ in significant ways. Neither BMP nor TBNPA have direct data suggesting they are a known or presumed human carcinogen. In addition to the multiple species, multiple tumor sites, the genotoxicity of BMP (in vitro and in vivo) added the strength of evidence justifying the classification of category 1B. Genotoxic carcinogens tend to cross species lines and represent a potential human hazard. However, this is not the case with TBNPA, therefore, the classification of TBNPA should be no greater than as Category 2.

Moreover, a 13-week repeated dose oral toxicity study for TBNPA, was initiated per ECHA's decision # CCH-D-2114381478-36-01/F and is currently ongoing. Previously, it was found, that the kidney pathology in the 28-day toxicity study of TBNPA was different than the kidney pathology in the BMP 90-day toxicity study. A direct comparison is confounded by the different durations of the two studies

It will be clear if TBNPA and BMP are similar after this study is done, and hence we ask to postpone the classification for carcinogenicity until the study is completed within 7 months' time.

In the attachment the rational for the above statements have been given in detail referring to the CLH report as such, starting from Page 18, III Carcinogenicity

ECHA note – An attachment was submitted with the comment above. Refer to public attachment TBNPA comments to CLH 23092019.pdf

Dossier Submitter's Response

Thank you for the comments and for informing us about the coming 90-day study. We now recognize that in the decision you have the requirement to submit the study and update the registration dossier by 25 March 2020. It is up to ECHA to decide whether to postpone the discussion until the study is available, but we agree that it seems reasonable to wait for this as it improves the database.

Regarding your comments to table 10 and mutagenicity, see comment number 6 and our response.

p. 19: In our view the additional bromine in TBPA would be expected to further increase the reactivity towards DNA compared to BMP.

We agree that there are differences in the physicochemical properties. These are shown in table 11 in the CLH proposal. Other uncertainties for the read-across approach is the lack of data on toxicokinetics for the target substance. In this case the source and target substance are structurally very similar, only one bromine molecule/hydroxyl group separates them and they belong to the same QSAR-based cluster for carcinogenity and mutagenicity (Wedebye et al., 2016). There is until now little experience with applying positive read-across for CLH proposals. We expect that the further process will set the bar for the requirements to similarity of physicochemical properties and have no further comments.

AE A.3: The test material in the NTP study for BMP was the commercial flame retardant FR-1138. We agree that the purity of the testmaterial should preferably have been higher and that the score should be set to 4 instead of 5.

AE 2.2/AE 2.3: We do not agree that the score for the Assessment Element (AE)2.2 Underlying mechanism, qualitative aspects and AE.2.3 Quantitative aspects should be reduced. Please see comment number 6 and our response.

AE 2.4: There is experimental support for oxidative DNA damage contributing to the induction of DNA damage and MoA of the carcinogenicity of BMP. Unmodified BMP seems to bind to DNA directly, a process that is more explicit in cells with low activity for glucuronidation, such as bladder cells (target) that in cells with high glucuronidation activity, such as liver cells (non-target). The DNA adducts formed are not identified. For more details, please see annex I: Consideration of two proposed hypotheses of mode of action for the carcinogenic effect of BMP. The annex was written for the development of the Risk Management Option Analysis for BMP in 2018 and includes newer references than the NTP study. We disagree that score 3 is too high.

AE 2.5: Concerning genotoxicity, please see comment number 6 and our response. Concerning oxidative DNA damage, please see comment to AE 2.4 above.

p. 21: The cancer models are commercial and the training sets are therefore proprietary and cannot be disclosed. However, QSAR systems need evidence from more than two compounds to make statistical significant alerts. The evidence we have at this point for chain alkyl molecules with bromide and hydroxyl group does in our opinion seem to alert strongly for genotoxic cancer, at least with when there's not steric hindrance.

In our view TBNPA should be classified in Carc. 1B, based on all relevant available information.

RAC's response

Experimental data addressing the carcinogenicity of TBNPA were not available. However, RAC accepts that data from studies with BMP can be read across for this endpoint

BMP was recently evaluated by RAC and was classified as Carc. 1B based on multi-site tumours in two species, rats and mice, with human relevance, in the presenc e of limited general toxicity. The opinion was adopted by consensus on June 8, 2018.

Briefly, from the BMP ODD "CLH-O-0000001412-86-212/F", *BMP induced dose-dependent multi-site tumours in two species, rats and mice, in a well conducted OECD TG 453 oral study carried out by the NTP under GLP conditions and with limited general toxicity. Both benign and malignant tumours were observed in the respective tissues, showing the ability of the tumours to progress to malignancy. The stop-exposure group in male rats showed that only 3 months of exposure induced tumours at most sites where tumours were observed in the 2-year continuous-exposure groups. The incidences of neoplasms were greater at some sites (lungs, small and large intestine, thyroid). Adenoma and carcinoma of the seminal vesicle were also found, which did not occur in the other groups, and which are extremely rare in rats. Based on the findings from this group, genetic damage appears to occur within the first few months of exposure and that can develop into tumours, also in the absence of a toxic response in these tissues. Some of the tumours observed fit into the pattern of genotoxic chemicals (NTP, 1996).*

In the table below a summary of the tumours observed in rats and mice in the source substance, BMP, is shown.

Table Tumours observed in the available studies for BMP

	BMP (NTP study; oral administration); (study conducted by industry, oral administration)			
Site	**Rats**		**Mice**	
	Male	**Female**	**Male**	**Female**
Skin	+			
Subcutaneous tissue	+			?
Nose				
Mammary gland	+	+		?
Zymbal's gland	+			
Oral cavity - Oral Mucosa	+	+		
Oesophagus	+	+		
Forestomach	+		+	?
Small intestine	+			
Large intestine	+			
Mesothelium - Peritoneum	+			
Liver				
Kidney	±		+	
Urinary bladder	+			
Lung	+		+	+
Spleen				
Thyroid gland	+	+		
Seminal vesicle	+	NA		NA
Tunica Vaginalis		NA		NA
Clitoral Gland	NA		NA	

Haematopoietic system	+			
Pancreas	?			
Harderian gland			+	+
Circulatory system				?

+: positive
?: equivocal results
NA: not applicable

It is apparent that the source substance, BMP, is a multi-site, multi-species carcinogen, as tumours were observed in both sexes of rats and to a lesser extent in mice.

Date	Country	Organisation	Type of Organisation	Comment number
17.09.2019	Germany		MemberState	3

Comment received

According to the CLP Regulation 1272/2008 (Section 3.6.2.2.7.), "a substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Cate-gory 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites". However, as the classification for carcinogenicity in the current proposal is assumed to rely on mutagenic activity, the uncertainties associated with the in vivo genotoxicity data contradict the classification of TBNPA as a genotoxic carcinogen, Category 1B. Furthermore, additional uncertainties regarding the robustness of the read-across (lack of toxicokinetic data for the target substance, differences in physicochemical properties, lack of comparable data regarding reproductive toxicity), which have not been discussed in the proposal, further raise doubts concerning the validity of the approach for classification of TBNPA.

References:
- NTP (1996): Toxicology and carcinogenesis studies of 2, 2-bis (bromomethyl)-1, 3-propanediol (CAS No. 3296–90-0) in
F344/N rats and B6C3F1 mice (feeding studies): Technical Report Series No. 452. US Department of Health and Human
Services. Public Health Service, National Institutes of Health, Research Triangle Park, NC

Dossier Submitter's Response

Thank you for your comments.

Regarding mutagenicity, please see comment number 6 and our response.

Regarding the robustness of the read-across, please se comment number 2 (with attachment) and our response.

RAC's response

See response to comment #2 above.

Date	Country	Organisation	Type of Organisation	Comment number
20.09.2019	France		MemberState	4
Comment received				
Based on the read across from the source substance BMP classified as Carc. 1B H350, we agree with the same classification for TBNPA.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Netherlands	ICL Europe Cooperative U.A.	Company-Manufacturer	5
Comment received				
ICL believes that the proposed hazard category for germ cell mutagens 1B is too severe based on the TBNPA database. TBNPA and BMP share similarities in the in vitro mutagenicity assays but divergent results in the in vivo tests. BMP is positive in vivo. All experimental data indicates that TBNPA is not an in vivo genotoxin. This is a significant difference. It has immediate impact on the weight of the evidence approach that is needed in the assessment of the germ cell mutation hazard and in the subsequent classification. For the purpose of establishing hazard assessments for genotoxicity, the battery conducted for TBNPA is sufficient without the necessity of using a (Q)SAR approach which assumes a paucity of available data. The actual TBNPA genetic toxicity data negates the reliance on the (Q)SAR model prediction. Although a (Q)SAR approach comparing BMP and TBNPA appears reasonable, the specific genotoxicity data does not support its use in the classification of TBNPA germ cell mutagenicity. ICL strongly believes that the classification has to be removed based on the lack of in vivo genetic damage shown experimentally, the differences between TBNPA and BMP with respect to in vivo genotoxicity and a weight of the evidence approach to the TBNPA genotoxicity battery. In the attachment the rational for the above statements have been given in detail referring to the CLH report as such, starting from Page 2, II Germ Cell mutagenicity ECHA note – An attachment was submitted with the comment above. Refer to public attachment TBNPA comments to CLH 23092019.pdf				
Dossier Submitter's Response				
Thank you for your comments. We disagree with your conclusion that TBNPA should not be classified for mutagenicity. Please see comment number 6 and our response to that comment. Response to your public attachment TBNPA comments to CLH 23092019.pdf: We appreciate your submission of the detailed data on the Ames test that was not already available to us (Table 1 in the attachment). We see that it could have been useful to request full study reports for the non-published in vitro studies and will do this as a follow-up to the further process. p. 3: We are aware that the sensitivity of different strains have been discussed, e.g. by Mortelmans & Zeiger: "Also, it has been shown [15] that the two-fold rule may be too insensitive for Salmonella strains with relatively high reversion frequencies, such as TA100, TA97, and TA102,				

and too sensitive for chemicals with low reversion frequencies, such as TA1535 and TA1537" (Mortelmans & Zeiger, 2000). Based on this we still consider the results of the Ames test to be positive in both mutation tests, but only in the highest concentration for TA 100. For TA 1535 we assume that the information in the registration still holds: "positive only in the presence of hamster S-9 mix (15-500 µg/plate)".

p. 6-7: We agree that BMP has not been evaluated in an in vitro mammalian cell gene mutation test (OECD TG 476), as shown in table 10 in the CLH proposal.

p. 10: Concerning the in vivo micronucleus test with TBNPA we thank you for submitting the blood plasma results that indicate bioavailability. We have not assessed the acute toxicity in our proposal, but based on the information in the ECHA dissemination site it seems to be low. See also our response to comment number 6 for additional arguments.

p. 12-13: Concerning the in vivo unscheduled DNA synthesis assay and the comparison of TBNPA with BMP in vivo responses, see our response to comment number 6.

p. 15: Note: We agree that the results on the germ cells in the 28-day toxicity study should be included in the database for genotoxicity and that **no** treatment related changes in sperm count and motility were observed.

p. 17: We disagree that the testbattery for TBNPA is sufficient without using a (Q)SAR approach, see comment number 6 and i.a. the comment from DE on the guidance on IR CSA R.7a, and our response.

In the attachment there is a detailed description of the studies and the test methods. We have no further comments to this information.

RAC's response

Based on the whole data set available for TBNPA the following findings can be summarised:

1. TBNPA was mutagenic in mouse lymphoma cells *in vitro* in the presence of metabolic activation.
2. TBNPA was clastogenic in human lymphocytes *in vitro* in the presence of metabolic activation and at the highest test concentration without metabolic activation is consistent with the mouse lymphoma test findings.
3. In bacterial reverse mutation assays, mutagenicity was seen. Nevertheless, the intensity of the positive results were debated by industry during the CLH consultation (cf. "Public Attachments" below).
4. Two *in vivo* tests with TBNPA were negative: a) in rat hepatocytes (UDS test) and b) micronucleus test in femur bone marrow cells of the mouse.
5. Regarding the micronucleus test in femur bone marrow cells, in order to conclude that a substance is clearly negative, it has to be demonstrated that the bone marrow had been exposed (adequate evidence of target tissue exposure). To this end, concentrations of the test item can be determined in the blood plasma. Industry provided data on the analysis of the blood plasma of animals treated with 300 mg/kg bw/d (maximum dose tested): 1h after treatment the plasma of the animals contained between 38.7 and 65.6 ng test item per mL plasma. The samples from the 4h interval did not have any detectable levels of the test item. In addition, industry stated that TBNPA did not induce any cytotoxic effects, as determined by the ratio between polychromatic and normochromatic erythrocytes, without providing actual data. A sound conclusion as to whether these values indicate systemic exposure including the bone marrow is questioned. Furthermore, acute toxicity testing at and above 500 mg/kg bw resulted in the death of some animals after 48 hours.
   In RAC's opinion, there are several limitations with the micronucleus test in femur bone marrow cells. Since 4h after exposure there is no TBNPA present in blood plasma, administration of the test substance could have been done differently. One variation according to OECD TG 474 is to administrate the test chemical as a split dose, i.e., two or more treatments on the same day separated by no more than 2-3 hours. A second variation

is to administrate the test item in more than one daily treatment for a more efficient exposure. Both of these variations could have improved the quality of the study.
In addition, industry stated that acute toxicity testing at and above 500 mg/kg bw resulted in the death of some animals after 48 hours. However, this result is equivocal since in the 90-day repeated dose toxicity study the top dose of 450 mg/kg bw/d was well tolerated with no acute, systemic or mortality effects observed. Moreover, the LD_{50} is > 2000 mg/kg bw for TBNPA so the dose selection for the specific study can definitely be disputed.
As assessed and explained in the RAC GENERAL COMMENT SECTION, RAC believes that the read across among the three SBAA is justified. However, RAC disagrees with industry's conclusion that the mutagenic profiles of TBNPA and BMP are different based on the comparison of the available *in vivo* micronucleus studies for TBNPA and BMP. The reasoning behind this argument is based on the substantially different parameters of the available studies.
In the mouse bone marrow micronucleus tests with TBNPA, the test substance was administrated in a single dose (top dose of 300 mg/kg bw/d) orally although it is not clear whether the route of exposure was gavage or feed (industry/gavage, CLH report/orally, CSR/feed). In the key positive study with BMP, significant increases in micronucleated normochromatic erythrocytes were observed in peripheral blood samples obtained from male and female mice exposed for 13 weeks to BMP in feed. These increases were seen in the two highest dose groups of male mice (1300 and 3000 mg/kg bw/d) and the three highest dose groups of female mice (600, 1200 and 2900 mg/kg bw/d). It is apparent that the exposure is much higher in the BMP experiment because of longer duration and higher dosing. In the first of two mouse bone marrow micronucleus tests performed with BMP, a three dose scheme was used with a top dose of 400 mg/kg bw/d with equivocal results as the first trial was negative and the second was positive. In this experiment the dosing is once more different that the one with TBNPA (3X400 vs 1X300 mg/kg bw/d). In the second mouse bone marrow micronucleus test, BMP was administered as a single intraperitoneal injection (150 to 600 mg/kg bw/d) and was positive with a significant dose related increase in micronucleated PCEs in females. In this study both the route of exposure (i.p. vs feed) and the dosing is different (600 vs 300 mg/kg bw/d). In conclusion, RAC believes that the micronucleus test in femur bone marrow cells with TBNPA has serious limitations and the comparison of the *in vivo* mutagenicity properties between TBNPA and BMP is not justified as the study parameters are not consistent across the studies.

6. According to ECHA guidance on Information Requirements and Chemical Safety Assessment – Chapter R.7a: Endpoint specific guidance (IR CSA R.7a), the use of the *in vivo* UDS indicator test should always be justified on a case-by-case basis and may only be sufficient under certain circumstances (considering target organ and substance-specific factors). Only if it can be reasonably assumed that the liver is a target organ, the UDS may be an adequate test. No available data indicate the liver to be the target organ. TBNPA is not expected to be highly metabolized in the liver, where glucuronidation activity (detoxification) is high (Risk Management Option Analysis for BMP in 2018; ECHA https://echa.europa.eu/documents/10162/a69d536b-4274-ff51-800a-65e6af17d0fa).
Furthermore, the guidance on IR CSA R.7a states that a negative result in a liver UDS test alone cannot be considered proof of absence of gene mutation inducing properties of the substance, despite the fact that based on the strain specificity observed in the *Salmonella typhimurium* assay, the UDS assay should have detected this type of gene mutation if it was occurring *in vivo*.
7. There is no indication that TBNPA reaches the germ cells, albeit the available database is limited.

Therefore, since positive results with TBNPA have been obtained from *in vitro* studies addressing both gene mutations and chromosome aberrations a relevant *in vivo* follow-up test is necessary to find out whether the *in vitro* results are also relevant *in vivo*. Negative results provided by the *in vivo* micronucleus test may indicate that TBNPA does not induce chromosome aberrations. However, uncertainties arise regarding dose scheme selection and the availability of the test substance at such doses. In addition, the fact that liver has not been proven to be a target organ renders the results from the UDS test questionable. Hence, as it cannot be ruled out that TBNPA has the potential to generate gene mutations *in vivo*, RAC recognises a data gap for the induction

of gene mutations *in vivo* and read across from BMP is applied. For BMP there is an adopted RAC opinion for germ cell mutagenicity 1B.

According to the CLP Regulation (Annex I: 3.5.2.2., note to table 3.5.1), classification as category 2 mutagens may be justified for substances "which are positive in *in vitro* mammalian mutagenicity assays", which is the case for TBNPA, and "which also show chemical structure activity relationship to known germ cell mutagens". This scenario can be considered to be relevant to TBNPA, where the results from the *in vivo* studies are not conclusive and read-across to a structurally related and known category-1B mutagen (BMP) is applicable. Overall, RAC considers classification in Category 2 justified.

Date	Country	Organisation	Type of Organisation	Comment number
17.09.2019	Germany		MemberState	6

Comment received

In the current CLH dossier, the DS proposes a classification of TBNPA concerning germ cell mutagenicity. The proposal is based on positive results from in vitro studies with TBNPA (target substance) in combination with read-across from the source substance 2,2-bis(bromomethyl)propane-1,3-diol (BMP) which has already been classified as mutagenic and carcinogenic Category 1B.

Positive results have been obtained in all three available in vitro studies following standard test guidelines (OECD TG 471, 473, 476) with TBNPA. Accordingly, TBNPA induces gene mutations in bacteria and gene mutations and chromosome aberrations in mammalian cells. In addition, two in vivo studies are available, showing negative results. An in vivo mammalian cytogenicity / erythrocyte micronucleus test (OECD TG 474 with GLP; reliability 1) conducted with TBNPA did not show induction of micronuclei in murine bone marrow cells. An indicator test concerning the induction of unscheduled DNA synthesis (UDS; TG OECD 486 with GLP) in rat liver cells indicated no TBNPA-mediated induction of DNA damages followed by DNA repair. No major deviations or shortcomings are reported within the CLH dossier. The validity of the two in vivo tests had not been questioned in any section of the dossier. Hence, the DS seems to consider the tests and the results as sufficient and reliable information.

Beyond the available studies conducted with TBNPA, the described rationale for proposal for classification in Cat. 1B is based on read-across from data obtained with 2,2-bis(bromomethyl)propane-1,3-diol (BMP; EC 221-967-7). The DS thereby refers to a Category approach established by the Danish Environmental Protection Agency (Wedebye et al., 2016). The analogue approach is further supported by providing data matrices for both TBNPA and BMP regarding available studies and physicochemical properties. The assessment of the analogue approach was done in line with scenario 2 of ECHA's Read-Across Assessment Framework (RAAF). As mentioned above, BMP has already been classified as being mutagenic and carcinogenic, Category 1B.

Validity of the application of a read-across approach – data gap filling unnecessary
The DE CA does not generally reject the Category approach established by the Danish Environmental Protection Agency and applied by the DS within the current proposal, but expresses concerns regarding the validity of the application of a read-across approach for classification for germ cell mutagenicity. According to ECHA's RAAF, read-across is an alternative approach for data gap filling using information from an analogous substance (source substance) to predict toxicological properties for the target substance. As data for the target substance are available, read-across may be an insufficient mean for the purpose of classification for germ cell mutagenicity. The CLP Regulation stipulates that read-across may only be applicable for classification in case evidence of genotoxicity is solely available from in vitro studies in addition to a chemical structure activity relationship to a known germ cell mutagen. This may lead to classification in Category 2.
Similar toxicological properties with regard to in vivo mutagenicity in somatic cells are not supported by data

On page 15, the DS states that TBNPA and BMP have almost identical genotoxicity test results. However, this holds true only for the available in vitro study results. The results of the available in vivo studies are contradictive. While a positive in vivo micronucleus test and a positive in vivo comet test (urinary bladder) with the source substance BMP provide sufficient evidence for BMP-mediated genotoxic effects in somatic cells, two negative in vivo studies with TBNPA are available and indicate no genotoxic potential in somatic cells. The DS does not discuss this inconsistency. If the available data with TBNPA provide sufficient evidence to conclude that TBNPA does not induce mutations in vivo, classification as germ cell mutagen may not be justified.

Other remarks on the robustness of the read-across approach
According to the PubChem Substance database, the chemical structure for CAS 36483-57-5 is unclear which may have an impact on the comparability of the source and target structure. Data on toxicokinetics are only available for the source substance. Similarities concerning absorption, distribution, metabolism and excretion cannot be compared between the target and the source substance. According to table 11 of the CLH dossier, differences in physico-chemical properties have been identified. Water solubility and Log Kow values, in particular, are clearly different between the source and the target substance which may have a profound effect on toxicokinetic properties of the substances. As indicated in the CLH dossier, reproductive toxicity studies indicating that TBNPA reaches the germ cells are not available. A thorough discussion of uncertainties associated with the approach is deemed necessary.

Critical assessment of the relevance and adequacy of available information regarding in vivo mutagenicity in somatic cell with the target substance
Given the existence of negative and therefore inconsistent data with TBNPA, the DS should provide a scientifically credible explanation as to why the negative results with the target substance do not compromise the read-across approach. With respect to the assessment of in vivo mutagenicity in somatic cells, clarification as to whether the DS considers the in vivo study results with TBNPA as less relevant compared to the results obtained with BMP or not relevant at all. As part of a weight of evidence approach, the available information should be assessed according to the quality of the data, consistency of results, nature and severity of effects, and relevance of the information for the final endpoint conclusion.

For instance, the highest dose in the in vivo micronucleus test with TBNPA was 300 mg/kg bw/d as compared to 400 mg/kg bw/d in the equivalent test (murine bone marrow micronucleus test by oral gavage) with the source substance (NTP, 1996; Unnamed, 2007). As positive results have only been reported for the highest dose in one out of two trials of the latter study with the source substance, the highest dose utilized in the in vivo micronucleus test with TBNPA may have been too low to induce effects. This is supported by the results of the second micronucleus test with the source substance (micronuclei in peripheral blood in mice treated with BMP in feed for 13 weeks; significant effects were observed at ≥ 1300 mg/kg bw/d in males and ≥ 600 mg/kg bw/d in females). Severe toxicity (death of one male mouse after 48h) has been observed following the application of 400 mg/kg bw/d by gavage in the pre-experiment of the in vivo micronucleus test with the target substance and in the first trial of the NTP in vivo micronucleus test with the source substance. Furthermore, to conclude that a substance is clearly negative regarding the induction of micronuclei in murine bone marrow cells, it has to be demonstrated that the bone marrow had been exposed (adequate evidence of target tissue exposure). To this end, concentrations of the test item can be determined in the blood plasma. Information on that is available for the in vivo micronucleus test with TBNPA (see REACH registration), indicating relatively high values (unit not indicated) in untreated animals that are only slightly lower as compared to the treatment group. Neither a unit for the values, nor proper statistics are given which does not allow for a sound conclusion as to whether these values indicate systemic exposure including the bone marrow. In addition, cytotoxic effects, as determined by the ratio between polychromatic and normochromatic erythrocytes, have not been reported. If it is not possible to convincingly demonstrate that bone marrow exposure to the substance occurred, the relevance of the negative result may be questioned.

Hence, the differences in the protocol of the in vivo cytogenicity tests between the source and the target substance and potential shortcomings are important elements to conclude on the relevance of the test result for the overall conclusion of whether or not the substance is clastogenic in vivo.

The potential of TBNPA to induce gene mutations in vivo has been addressed with an in vivo UDS test (OECD TG 486). The results are negative. The relevance of the in vivo UDS test for the overall conclusion on the genotoxic potential of the target substance has not been discussed within the CLH dossier. According to the guidance on Information Requirements and Chemical Safety Assessment – Chapter R.7a: Endpoint specific guidance (IR CSA R.7a), the use of the in vivo UDS indicator test should always be justified on a case-by-case basis and may only be sufficient under certain circumstances (considering target organ and substance-specific factors). Only if it can be reasonably assumed that the liver is a target organ, the UDS may be an adequate test. However, the results of the in vivo comet assay with the source substance do not indicate genotoxicity in the liver. Furthermore, the guidance on IR CSA R.7a states that a negative result in a liver UDS test alone cannot be considered proof of absence of gene mutation inducing properties of the substance. Further evidence is needed to assess the ability of the TBNPA to induce gene mutation in vivo. The endpoint gene mutation in vivo is therefore not adequately addressed which may justify the application of read-across.

Nature of the mutagenic effect
Positive results with TBNPA have been obtained from in vitro studies addressing both gene mutations and chromosome aberrations. Consequently, relevant in vivo follow-up tests are necessary to find out whether the in vitro results are also relevant in vivo. Negative results provided by the in vivo micronucleus test may indicate that TBNPA does not induce chromosome aberrations. However, due to the uncertainties associated with the available UDS indicator test, it cannot be ruled out that TBNPA has the potential to generate gene mutations in vivo. Hence, there may be a data gap for the induction of gene mutations in vivo. However, as the in vivo comet assay, conducted with the source substance BMP, does not allow a definitive conclusion on the nature of the mutagenic effect (DNA damages may or may not lead to gene mutations and/or chromosomal aberrations), it cannot be concluded that the source substance induces gene mutations in vivo. Thus, the positive result of the in vivo comet assay may not be considered sufficient information to predict the potential of the target substance with respect to the genotoxic endpoint gene mutations in vivo.

According to ECHA's PACT, TBNPA will be addressed as part of a substance evaluation by the DK CA in 2021 (suspected carcinogenic/mutagenic). Additional study results may arise as a consequence of the SEv.

Conclusion
Hazard categories for germ cell mutagens according to CLP Regulation 1272/2008 (Table 3.5.1):
Category 1A
The classification in Category 1A is based on positive evidence from human epidemiological studies.

Category 1B
The classification in Category 1B is based on:
- positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the
substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from
mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s)
to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of
transmission to progeny; for example, an increase in the frequency of an-euploidy in sperm cells of exposed people.

Category 2:
The classification in Category 2 is based on:
- positive evidence obtained from experiments in mammals and/or in some cases from in vitro

experiments, obtained from:
- somatic cell mutagenicity tests in vivo, in mammals; or
- other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

The DE CA agrees that classification in Category 1A is not justified. However, the DE CA disagrees with the proposed classification in Category 1B for the following reason. As indicated above, classification in category 1B is based on positive in vivo results in mammals. No such information has been identified. Hence, classification in Category 1B is not justified.
According to the CLP Regulation, classification in Category 2 (suspected Category for herita-ble germ cell mutagens) may be justified for substances 'which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens'. This scenario may be applicable if no in vivo studies for TBNPA are available. However, two in vivo studies with TBNPA showing negative results are available.

Only if it can be demonstrated that the available in vivo information is insufficient to conclude that TBNPA does not induce mutations in vivo (e.g. due to shortcomings and uncertain-ties such as the dose selection in the in vivo micronucleus test or the general validity of the UDS test), classification in Category 2 may be justified base on positive in vitro data and read-across to BMP.

References:
- Unnamed (2007): Study Report. https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/3/?documentUUID=ca22dcb7-3231-4dd3-95f8-44fad85f4c68
- Wedebye E.B., Nikolov N.G., Nielsen E.E., Boberg J., Petersen M.A., Reffstrup T.K., and Dybdahl M. (2016): Category approach for selected brominated flame retardants: Preliminary structural grouping of brominated flame retardants

Dossier Submitter's Response

Thank you for your detailed and helpful comments.

See also our response to comment no. 2 on uncertainties associated with the read-across approach.

It is correct that the the proposal is based on positive results from in vitro studies with TBNPA (target substance) in combination with read-across from the source substance 2,2-bis(bromomethyl)propane-1,3-diol (BMP).

We agree that reproductive toxicity studies indicating that TBNPA reaches the germ cells are not available and that this should have been mentioned.

We agree that the inconsistency in the in vivo results was not discussed in the proposal. We recognize that there are differences between the target and the source:

In the in vivo micronucleus test with TBNPA we agree with you reasoning and the conclusion that the highest dose may have been too low to induce effects. In addition negative results in rodent micronucleus test are not good predictors of noncarcinogenicity (NTP TR 587, 2014).

We can not find the information on blood plasma in the registration that you refer to. However, ICL Europe Cooperative U.A. has submitted this information about the micronucleus test on TBNPA, in their attachment (p.9) to their comment: "The analysis of the blood plasma of animals treated with 300 mg test item per kg b.w. showed, that 1 h after treatment quantifiable amounts of the test item could be detected. The plasma of the animals contained between 38.7 and 65.6 ng test item per mL plasma. The samples from the 4 h interval did not have any detectable levels of the test

item. FR-513 did not have any cytotoxic effect on the bone marrow as assessed by PCE/NCE ratios". This may indicate that the systemic exposure includes the bone marrow.

Based on the Comet assay and carcinogenicity study with the source substance and supported by studies of repeated dose toxicity of both source and target substance it seems that the liver is not a target organ. So we agree that the UDS test may not be adequate for TBNPA. We also agree that the positive result of the in vivo Comet assay for the source substance does not allow a definitive conclusion on the nature of the mutagenic effect (neither structural chromosomal nor mutation is detected directly). The UDS test response positively only to chemicals that induce the type of DNA damage that is repaired by nucleotide excision repair.

After reconsidering the available information, we agree that the note in CLP section 3.5.2.2. Table 3.5.1 can be applied:

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

Based on the above and the QSAR clustering for genotoxicity (BMP and TBNPA in same cluster, Wedebye et al., 2016) we have revised our conclusion and now propose TBNPA to be classified in Category Muta. 2.

RAC's response

RAC agrees to classify TBNPA as germ cell mutagen category 2. For more details see responses to comments #5 for mutagenicity reasoning and #1 for read-across reasoning.

Date	Country	Organisation	Type of Organisation	Comment number
20.09.2019	France		MemberState	7

Comment received

P8:
In the chromosome aberration study in peripheral human lymphocytes, the use of mitomycin C (active clastogen without metabolic activation) as positive control does not demonstrate the effectiveness of the exogenous metabolic activation system employed in the test.
This study and the one following OECD TG 476 did not mention the presence or absence of a dose-response relationship. Could you precise if this information is available?

P10:
The negative findings in the two in vivo studies have not been discussed. It should have been considered whether there has been adequate evidence of target tissue exposure.

According to the guidance ("Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens"), the positive results in the in vitro mutagenicity studies and the read across from the source substance BMP known as germ cell mutagen, should lead to a classification as Category 2 mutagen rather than Category 1B for TBNPA.

Dossier Submitter's Response

Thank you for your comments.

P8: Unfortunately, the results in the registration are not presented in such a detail that the dose-response relationship for mutagenicity can be described. We will request full study reports for the non-published in vitro studies.

P10: See comments 6 and our response to that.

RAC's response
RAC agrees to classify TBNPA as germ cell mutagen category 2. For more details see responses to comments #5 for mutagenicity reasoning

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	Sweden		MemberState	8

Comment received
Considering the structural similarity between TBNPA and BMP and that the additional bromine in TBPA would be expected to further increase the reactivity towards DNA, we agree that read-across from BMP for potential mutagenic properties of TPNPA seems appropriate. However, we do not consider the justification for read-across presented emphasizing structural similarity, similar physicochemical properties and results in genotoxicity tests fully convincing. According to table 13 the two substances share the same genotoxic properties and give similar genotoxic responses (assessment given score 4 of 5). However, this does not seem fully supported from the data in table 10. While the limited amount of studies with TBNPA compared to BMP complicates the assessment it is still clear from the data matrix in table 10 that the in vivo micronucleus test with TBNPA resulted in a negative result whereas a positive result was obtained in the same study with BMP. Furthermore, although not a sensitive assay, a negative result was obtained in the in vivo UDS assay with TBNPA whereas a positive result was obtained with BMP in an in vivo comet assay. Finally, impaired fertility and decreased counts of growing follicles were observed in a continuous breeding assay in mice with BMP whereas no effects on (female) germ cells were observed in the 28-day study with TBNPA in rat. Annex I: 3.5.2.2. states in a note to table 3.5.1: "Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens." Therefore, although we do not disagree with the read-across hypothesis we think the different results obtained with the target and source substances in the genotoxicity tests need to be further discussed to support read-across from BMP and classification in Muta. 1B.

Dossier Submitter's Response
Thank you for your comments. We agree that the additional bromine in TBPA would be expected to further increase the reactivity towards DNA. Regarding your other comments on the read-across, please see our response to comment number 2. Regarding your comments on genotoxicity, please see our response to comment number 6.

RAC's response
RAC agrees to classify TBNPA as germ cell mutagen category 2. For more details see responses to comments #5 for mutagenicity reasoning and #1 for read-across reasoning.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Netherlands	ICL Europe Cooperative U.A.	Company-Manufacturer	9

Comment received

ICL agrees with the 14 June 2019 CLH report that there are no effects on developmental or germ cells that warrant classification for reproductive toxicity. However, several inconsistencies in data reporting and conclusion in the 14 June 2019 CLH report are present. A toxicity study evaluated many germ cell parameters and found no effect of TBNPA on these parameters. A prenatal developmental study that evaluated fertility, number of implantations, resorptions, live young and percentages of sex ratio and pre- and post- implantation loss found no effects.
These studies were identified and described in separate sections of the CLH report but clarifications are needed in the final document on classification. Conclusions in some sections state the data is considered 'inconclusive' and in others 'not considered sufficiently severe to meet the criteria for classification'. ICL kindly request the references to the classification of reproductive toxicity to be uniformly expressed as 'not considered sufficiently severe to meet the criteria for classification

ECHA note – An attachment was submitted with the comment above. Refer to public attachment TBNPA comments to CLH 23092019.pdf

Dossier Submitter's Response

Response to the attachment:
p. 21-22: Concerning the prenatal developmental toxicity study: In GD IR & CSA R.7a p. 499 it is stated that: "It should be noted that a prenatal developmental toxicity study (EU B.31, OECD TG 414) does not provide information on postnatal development or sufficient information on female fertility. However, some findings might raise concerns; if exposure started on gestation day 0, effects on preimplantation or implantation could indicate effects on female fertility. Also effects on maintenance of pregnancy and potentially on gestation length may be identified if significantly affected." In this study exposure started on GD 6 so implantation could not indicate effects on female fertility. The other parameters that you mention, such as resorptions etc are parameters for offspring developmental toxicity and not for fertility.

To your last comment on p. 22, please note that TBNPA and BMP are in different clusters based on reproductive toxcity QSAR-based clustering (Wedebye et al., 2016).

p.23: In the classification system reproductive toxicity is subdivided into adverse effects on a) sexual function and fertility and b) development of the offspring. We agree that for developmental toxicity the conclusion could possibly be changed to "data conclusive but not sufficient for classification". However, for fertility, we still think the data is inconclusive. Overall, for reproductive toxicity the conclusion remains the same i.e. "data inconclusive".

RAC's response

For read-across see response to comment #1.

Regarding data on fertility, RAC gathered the following information:

In the OECD TG 414 pre-natal developmental toxicity study in female SD rats (20/dose), carried out as part of the ECHA decision number TPE-D-21 I43LO292-65-OUF (2015), minor ossification effects were observed, along with lower gravid uterine weight in the dosed animals. No systemic toxicity was noted.

In the 28-day repeated dose toxicity study, no treatment related changes in sperm count and motility were observed. Vaginal lavages, which were taken early morning during the 3rd week period from all females, prior to termination of the animals showed no treatment related changes in the oestrus cycle. In addition, no treatment related changes in the weight of seminal vesicles, ovaries, testes, ureter, uterus and vagina were observed.

On 23rd March 2020, an oral 90-day repeated dose toxicity study in rats (EU B.26./OECD TG 4O8) requested by an ECHA decision in 2017 (CCH-D-21 L43BI47B-36-0UF) was submitted via IUCLID and evaluated by RAC.

In the said study, TBNPA was administered by oral gavage to Sprague Dawley rats at 50, 150 and 450 mg/kg bw/d. No mortalities were observed up to the highest dose over the 90-day dosing period and the 28-day recovery period. The body weights, body weight gains and food consumption were not altered by the treatment at any of the doses tested in either sex.

Very few fertility parameters were evaluated in this study, like the oestrous cycle in females. More specifically, the stage of oestrous cycle was recorded prior to necropsy in the treated groups only to facilitate interpretation of ovary and uterus organ weight (no test item related changes in the organ weights reported) and histopathology. No intergroup differences were observed in either parameter. In the males, no significant intergroup differences in sperm motility, sperm morphology and sperm counts were observed.

An isolated incidence of dilated uterus one each in 50 and 450 mg/kg bw/d dose group female was observed and considered as incidental finding and not related to test item administration.

No hormone analysis related to reproduction was reported.

It is evident that the dataset for any possible fertility and developmental effects for TBNPA is limited. Although no actual findings were reported that could raise concern for either cluster of effects, for fertility the findings are not conclusive to decide for classification or no-classification. For developmental effects, the findings reported from the OECD TG 414 study in rats with TBNPA are not sufficient to trigger classification.

In addition, since in the OECD TG 414 study with TBNPA the exposure started on gestation day 6, no conclusions could be drawn on female fertility.

Hence, taking into consideration the scattered data on fertility that can be harvested from the 20-days and 90-days RTD studies with TBNPA, RAC concludes that classification of TBNPA for Reproductive Toxicity – Fertility is not warranted due to lack of data.

In addition, RAC concludes, based on OECD TG 414 prenatal developmental toxicity study for TBNPA, that classification of TBNPA for Reproductive Toxicity – Developmental effects is not warranted.

Date	Country	Organisation	Type of Organisation	Comment number
17.09.2019	Germany		MemberState	10
Comment received				
Effects on fertility of TBNPA have only been assessed with a 28-day repeated dose toxicity study. Some parameters relevant for fertility have been investigated (sperm count and motility, vaginal lavage, organ weight of ovaries, seminal vesicles, testis, uterus, vagina) and no effects identified. Based on the information available for TBNPA we agree with the DS that criteria for classification are not met for the fertility endpoint. Effects on developmental toxicity of TBNPA have been assessed with an OECD TG 414 study (rats, oral). No treatment related effects on development were identified. Based on the information available for TBNPA we agree with the DS that criteria for classification are not met for the endpoint developmental toxicity. For BMP, serving as source substance for the endpoint mutagenicity, a two-generation reproductive toxicity study is available. In this study, the reproductive performance of female mice was affected by treatment with the substance at the 0.4% dose level. Has a potential read across to BMP for the endpoint reproductive toxicity been considered by the DS?				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

Dossier Submitter's Response
Thank you for your support. Yes, read-across was considered, but not proposed as TBNPA and BMP are in different clusters based on reproductive toxcity QSAR-based clustering (Wedebye et al., 2016). Please see comment number 9 and our comments to that for more details.
RAC's response
RAC appreciates the suggestion to read-across from BMP. For more details see response to comment #1 for read-across approach in general and for reproductive toxicity response to comment #9.

Date	Country	Organisation	Type of Organisation	Comment number
20.09.2019	France		MemberState	11
Comment received				
We agree that the data regarding reproductive and developmental toxicity do not warrant classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comments. For developmental toxicity RAC agrees that no classification is warranted. For fertility effects, see response to comment #9.				

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	Sweden		MemberState	12
Comment received				
We were wondering, based on the read-across hypothesis and your justification of read-cross from BMP to TBNPA for carcinogenicity and mutagenicity based on structural similarity, whether read-across also for reproductive toxicity – fertility could be explored? Or at least perhaps it could be described better why it would not be appropriate to use supporting data from both BMP and 2,3-DBPA for assessment of adverse effects on fertility and sexual function. We do not consider that the three substances (TBNPA, BMP and 2,3-DBPA) being in separate QSAR-based clusters for reproductive toxicity to be a strong reason for excluding the possibility of read-across for this end-point. It would be better to compare the toxicity profile based on the actual data from available studies on reproductive toxicity.				
Dossier Submitter's Response				
Thank you for your comment. Se comment number 9 and 10 and our response to those. Regarding reproductive toxicity we are aware that there are some findings in a two-generation reproductive toxicity for BMP. These were not considered to warrant classification by the registrant. Our previous CLH-proposal for BMP was targeted to Muta. and Carc. Primarily based on an NTP-report. 2,3-DBPA has a harmonised classification as Repr. 2. TBNPA, BMP and 2,3-DBPA are in separate QSAR-based clusters for reproductive toxicity. An investigation of the toxicity profile for reproductive toxicity should start with scrutinizing the results of the two-generation reproductive toxicity for BMP. This would however probably not result in a stronger risk management of the substances.				

RAC's response
RAC appreciates the suggestion to read-across from 2,3-DBPA. For more details see response to comment #9.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
17.09.2019	Germany		MemberState	13

Comment received
Three repeated-dose toxicity studies are available for TBNPA (unnamed study reports from 1973 (30 days feeding study, rats, no guideline), 2011 (14 days oral gavage, rats, no guide-line), 2015 (OECD TG 407, rats)). Effects on liver (increased organ weight, minimal centrilobular hypertrophy), kidney (increased organ weight), and urine bladder (hyperplasia of the mucosal lining of urinary bladders) are documented. Most findings occurred above 100 mg/kg bw/d and showed full recovery. Thus no significant toxic effects were observed according to the Guidance on the Application of the CLP Criteria, except at 1000 mg/kg bw/d (unnamed study report, 2011), which is above the guidance value for category 2 classification. Therefore, we agree with the DS that criteria for classification are not met for the endpoint specific target organ toxicity-repeated exposure.
Dossier Submitter's Response
Thank you for your support.
RAC's response
RAC appreciates the comment. The recently submitted sub-chronic toxicity study (90-day) by oral route (EU B.26./OECD TG 408) in rats, as requested by ECHA (Anonymous, 2020) had the goal of to assess the systemic toxicity potential of TBNPA and to compare its toxicological profile and more specifically the kidney pathology of TBNPA with that of BMP. TBNPA was administered by gavage for 90 days in Sprague-Dawley rats, with a 28 day recovery period in order to access the reversibility of any effects observed. The doses of 50, 150 and 450 mg/kg bw/d resulted in no mortalities, no changes in haematology, coagulation parameters, thyroid hormone levels and urine parameters. There were no gross pathological changes observed at any of the doses tested. There were clinical signs of perineum wet with urine observed in both males and females which were fully reversed at the end of the recovery period. The main effects observed were in the kidneys and in the urinary bladder. These effects were correlated with blood urea and creatinine changes and with the clinical sign of perineum wet with urine. More specifically, at 150 mg/kg bw/d in males, an increase in creatinine correlated with increased eosinophilic droplets in tubular epithelium in kidneys and urinary bladder epithelial hyperplasia were considered. These were considered as test item related changes. At 450 mg/kg bw/d in males, a minimal increase in blood urea nitrogen and creatinine was correlated with morphological changes in the kidneys and the urinary bladder. In kidneys, increased eosinophilic droplets were noted in the tubular epithelium in the cortex of 6 males treated at 150 mg/kg bw/d and all males treated at 450 mg/kg bw/d but not in females. A single incidence of papillary necrosis was also observed at 450 mg/kg bw/d males and considered as test item related. Diffuse epithelial hyperplasia was noted in urinary bladder of 6 males at 150 mg/kg bw/d and all males and one female at 450 mg/kg bw/d. Both these changes reversed at the end of recovery period. Based on the above findings, treatment did not cause any adverse effects at 50 mg/kg bw/d in males and at 150 mg/kg bw/d in females during the 90 days treatment period. It is worth noting that at the highest dose (450 mg/kg bw/d) no signs of systemic toxicity were observed. It could be argued that a higher dosing schedule should have been applied in order to observe the full toxicological spectrum of TBNPA.

In the key experimental study (Anonymous, 2015), the oral gavage administration of TBNPA to Sprague-Dawley rats at doses of 30, 150 or 500 mg/kg bw/d for four weeks was well-tolerated and did not cause any adverse change. A substance related response was evident in the liver (predominantly at ≥ 150 mg/kg bw/d) as indicated by increased organ weight and a correlative microscopic finding of slight minimal centrilobular hypertrophy. Some changes in blood chemistry (low sodium and high potassium concentrations in males at 500 mg/kg bw/day) or urine composition/output (increased urinary volume and total protein and glucose output in males at 500 mg/kg bw/day) occurred and a slight increase in kidney weight was evident in both sexes (predominantly at ≥ 150 mg/kg bw/day). None of these changes were considered adverse in nature, however, and the majority showed full or at least partial recovery.

In the supporting 14-day repeated dose oral (gavage) toxicity study in rats (non-guideline, no GLP; Anonymous, 2011), administration of TBNPA to CD rats at doses up to 1000 mg/kg bw/d in females and 300 mg/kg bw/d in males was well tolerated and these doses are considered to be the NOAEL. However, doses of 1000 mg/kg/d in males necessitated premature sacrifice of these animals on day 4 and was considered to exceed the maximum tolerated dose.

In the supporting 30-day repeated dose oral (feeding) toxicity study in rats (non-guideline, no GLP; Anonymous, 1973), the ingestion of up to 30 mg/kg/d of TBNPA in the diet of Sprague-Dawley rats for 30 days did not cause changes in the toxicological parameters evaluated. At levels of 100 and 300 mg/kg/day, histologic changes in kidneys and urinary bladder were noted in male rats. No changes were noted in any of the female rats in this study.

Based on the available studies, it is apparent that repeated exposure to TBNPA targets primarily the kidneys (increased organ weight, increased eosinophilic droplets, papillary necrosis) and the urine bladder (hyperplasia of the mucosal lining of urinary bladders) and secondarily the liver (increased organ weight, minimal centrilobular hypertrophy). The effects in the kidneys and the urinary bladder are more of concern since they were also observed in the supporting study (Anonymous, 1973) and are accompanied by clinical findings and altered biochemistry. However, the findings in all studies were mild, reversible and observed at doses above the guidance values for classification (STOT RE 2 ≤ 100 mg/kg bw/d for 90-day study).

In conclusion, the available repeated dose toxicity data for TBNPA is adequate for evaluation and RAC bases the evaluation of the STOT RE endpoint on the TBNPA repeated dose toxicity studies, which provide a complete database for classification. In these studies, mild, reversible effects in the kidneys and urinary bladder were observed at doses above the guidance values for classification. Therefore, RAC considers that despite the fact that clinical signs (perineum wet with urine) and biochemistry (minimal increase in blood urea nitrogen and creatinine) support the histopathological observations, **no classification for STOT RE is warranted**, in agreement with the DS.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

Date	Country	Organisation	Type of Organisation	Comment number
20.09.2019	France		MemberState	14
Comment received				
P21-22: Please precise the number of animals used in the studies from 2011 and 1973. Based on the available data showing no significant toxic effects in the animals at low or moderate exposure concentrations, we agree with the proposal that no classification is warranted for TBNPA regarding STOT-RE.				
Dossier Submitter's Response				
Thank you for your comment and your support. In the 2011 study: • Crj: CD(SD) rats, male and female (a total of 25 male and 25 female) • 5 male and 5 female per dose In the 1973 study: • Male and female Sprague-Dawley rats • 5 animals per sex per dose				
RAC's response				
RAC appreciates the comment. For more details see response to comment #13.				

PUBLIC ATTACHMENTS

1. TBNPA comments to CLH 23092019.pdf [Please refer to comment No. 1, 2, 5, 9]

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2,2-DIMETHYLPROPAN-1-OL, TRIBROMO DERIVATIVE; 3-BROMO-2,2-BIS(BROMOMETHYL)PROPAN-1-OL

Annex I: Consideration of two proposed hypotheses of mode of action for the carcinogenic effect of BMP

In TR-452 from NTP on BMP two hypotheses for the carcinogenic activity of brominated chemicals are proposed: 1) bromine from the molecule causes oxidative damage to DNA and other cellular constituents and 2) the C-Br bond is broken and the remaining carbon-containing electrophilic group forms DNA adducts with subsequent DNA damage.

Experts at the Norwegian Institute of Public Health (NIPH) has evaluated the available scientific data regarding these two hypotheses in this short report, and concludes that neither hypotheses are supported with satisfactory evidence. However there is data demonstrating that BMP leads to the induction of oxidative DNA damage, which could be due to the release of bromine.

Metabolic activation of BMP does not seem to occur. The only known metabolic pathway is by UDP-glucuronosyltransferase (Ugt) glucuronidation. (Hoehle et al., 2009), which is highly active in the liver as compared to bladder. There has been no demonstration that BMP form reactive metabolite(s) in vivo. Moreover, no evidence for cytochrome P450-mediated mono-oxygenation or/and glutathione conjugation of BMP has been obtained (Hoehle et al., 2009).

DNA lesions are induced after BMP in organs and cell systems, but the specific origin of these DNA lesions is not clear. As demonstrated by Wada et al., 2014 in urinary bladders of SD-rats, BMP induced DNA damage in the conventional in vivo comet assay. Moreover, in mouse lung neoplasms induced by BMP G-A transitions were induced in the K-ras codon, demonstrating that Guanines are attacked leading to mutations and cancer (Ton et al.,2004).

There is experimental support for oxidative DNA damage contributing to the induction of DNA damage and MoA of the carcinogenicity of BMP. Unmodified BMP seems to bind to DNA directly, a process that is more explicit in cells with low activity for glucuronidation, such as bladder cells (target) that in cells with high glucuronidation activity, such as liver cells (non-target). The DNA adducts formed are not identified.

There are several lines of experimental evidence suggesting that oxidative damage is involved (Kong et al., 2011; 2013). First specific oxidative base modifications were demonstrated in human urothelial cells (UROtsa) exposed to BMP in vitro when a lesion specific endonuclease, the human 8-hydroxyguanine DNA glycosylase 1 (hOGG1) was introduced into the comet assay (Kong et al., 2011). Moreover, DNA strand breaks were attenuated when cells were pre-treated with the anti-oxidant N-acetyl-L-cysteine (Kong et al., 2011).

Evidence for BMP associated oxidative stress further include an elevation of intracellular ROS formation as well as induction of proteins involved in the response to oxidative stress, Nrf2 and HSP70 protein levels (Kong et al., 2011). Cells with high levels of Glutathione (GSH) (rat hepatocytes) reveal fewer DNA strand breaks (Comet assay) than cells with low GSH levels (human UROtsa cells) (Kong et al., 2013). This indicates that the ability of the tissue or cell system to counteract induction of oxidative damage reduces DNA lesions by BMP induced reactive oxygen species.

There is some evidence that BMP exposure disrupts the redox cycling and antioxidant enzyme systems in target cells. A number of antioxidant genes in bladders of B6C3F1 mice were up-regulated after 6 h of BMP dosing (300 mg/kg, PO) (unpublished results, Kong et al., 2013). The up-regulated genes include heme oxygenase (decycling) 1 (Hmox1, 46-fold), glutathione S-transferase (Gsta1 and Gsta2, 8-fold), glutathione synthetase (Gss, 5-fold), glutathione –cysteine ligase (Gclm, 5-fold), glutathione peroxidase 2 (Gpx2, 5-fold), thioredoxin reductase (Txnrd1, 5-fold), metallothionein 2 (Mt2, 5-fold) and glutathione reductase 1(Gsr1, 4-fold). As many of these genes are downstream targets regulated by transcription factor Nrf2, these data are consistent with the finding that Nrf2 was induced by BMP in UROtsa cells (Kong et al., 2011).

With regard to hypothesis 2 we found no specific evidence of C-Br bond breakage occurring in vivo, leaving this hypothesis quite speculative. No specific DNA adducts have been identified. However, Kong et al., 2013 suggests that the level of BMP-induced strand breaks measured in the human UROtsa cells is several fold lower than the amount of bound BMP to DNA. Furthermore, DNA damage (single-strand

breaks and alkali-labile sites) induced in the standard alkaline comet assay without enzyme inclusion could be the result of reactive metabolite generated from C-Br bond breakage. However, this cannot be specifically ascribed since both oxidative DNA damage and DNA adducts may give rise to increased levels of DNA damage detected in this assay. As mentioned in the TR-452 NTP report, Weiss et al. (1986) showed that eosinophils contain a lysosomal peroxidase that oxidizes bromide to a highly reactive oxidant such as hypobromous acid, but this is part of their role in inflammation processes and anti-parasitic functions. Lysozomal activity in eosinophils cannot be regarded as a general process in all cell types, and has almost no relevance for the effects observed in UROtsa cells and hepatocytes.

QSAR analysis of the molecule in VEGA (Benfenati et al, VEGA report) assesses BMP as mutagenic in five models, and carcinogenic in four models based upon available experimental data in the training set. The same models PREDICT mutagenicity and carcinogenicity with structural alerts from the C-Br structure and variants thereof (SA8 aliphatic halogens). This may be an ambiguous clue, as this would indicate that the C-Br bond needs to be broken for the genotoxic effect to occur.