Committee for Risk Assessment

RAC

Annex 1

Background document

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

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

Adopted

11 June 2020

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

EC Number: 253-057-0

CAS Number: 36483-57-5 and 1522-92-5

Index Number: 603-RST-VW-Y

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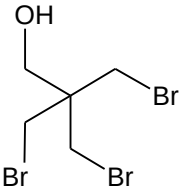
Version number: 1.2 **Date: 14 June 2019**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance. Taken from ECHA´s dissemination site if no other source is given

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol
Other names (usual name, trade name, abbreviation)	TBNPA, FR-513
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	253-057-0
EC name (if available and appropriate)	2,2-dimethylpropan-1-ol, tribromo derivative
CAS number (if available)	36483-57-5;1522-92-5
Other identity code (if available)	-
Molecular formula	$C_5H_9Br_3O$
Structural formula	
SMILES notation (if available)	BrCC(CBr)(CBr)CO
Molecular weight or molecular weight range	324.838 g/mol (from PubChem)
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
TBNPA (CAS no. 36483-57-5; 1522-92-5)	≥ 97% (w/w)	-	Aquatic chronic 3 H412 Eye Irrit. 2 H319 Acute Tox. 4 H302 Muta. 2 H341 Muta. 1B H340 Carc. 1B H350 Not classified

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Proposed CLH

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-										
Dossier submitters proposal		2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol	253-057-0	36483-57-5; 1522-92-5	Muta. 1B Carc. 1B	H340 H350	GHS08, Dgr	H340 H350			
Resulting Annex VI entry if agreed by RAC and COM		2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol	253-057-0	36483-57-5; 1522-92-5	Muta. 1B Carc. 1B	H340 H350	GHS08, Dgr	H340 H350			

Table 4: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has no previous harmonised classification and labelling

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

RAC general comment

2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol (TBNPA) is a small brominated alkyl alcohol. TBNPA is a REACH registered substance and according to the information published on the ECHA dissemination site, this substance is used in the manufacture of polymers, plastic products and chemicals, as well as an intermediate. TBNPA is a reactive flame retardant in polymer synthesis (100-1000 tonnes per year, tpa) for the manufacture of plastic products and chemicals. It is used in industrial, professional and consumer settings in formulation and use of commercial mixture(s). The substance currently does not have an Annex VI entry according to the CLP regulation. The dossier submitter (DS) evaluated Germ Cell Mutagenicity, Carcinogenicity, Reproductive Toxicity, Specific Target Organ Toxicity after Repeated Exposure and proposed classification as Carc. 1B and Muta. 1B.

In the CLH dossier, extensive use is made of read-across of data from another brominated alkyl alcohol, 2,2-bis(bromomethyl)propane-1,3-diol (BMP), recently evaluated by RAC (CLH-O-0000001412-86-212/F, adopted June 2018). The read-across of data is mainly based on a document by the Danish Environmental Protection Agency, which grouped several brominated flame retardants. RAC agrees with the proposed read-across and also extends it to include 2,3-dibromo-1-propanol (2,3-DBPA), which is a structurally similar brominated flame retardant with a harmonised classification and labelling. The reasoning for the use of read-across of data is explained and justified as follows.

Read-across

The toxicological data presented in the CLH dossier and/or available in the open literature for TBNPA are very limited and the available studies are the following (see more details in the table below):

1. a 28-day oral repeated dose toxicity (RDT) study in rats with a 14 day recovery period where some relevant reproduction parameters were investigated (Anonymous, 2015; REACH registration dossier)
2. an OECD TG 414 pre-natal developmental toxicity study on female Sprague-Dawley (SD) rats (20 /dose) requested by ECHA on 2015 (TPE-D-21 I43LO292-65-OUF), (Anonymous, 2016)
3. a 14-day repeated dose oral toxicity by gavage in rats used as supporting study in the evaluation of STOT RE (Anonymous, 2011)
4. a non-guideline, 30-day feeding study in rats used as supporting study in the evaluation of STOT RE (Anonymous, 1973)
5. Several *in vitro* mutagenicity studies in bacteria and mammalian cells and 2 *in vivo*

mutagenicity studies

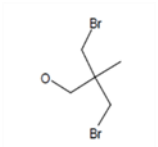
In addition, a sub-chronic toxicity study (90-day) by oral route (EU B.26./OECD TG 408) in rats was also requested by ECHA with a deadline of submission 25th March 2020 (CCH-D-21 L43BI47B-36-0UF). Industry submitted the study, which became available to RAC on 23rd March 2020.

Therefore, the need for identifying substances with similar chemical structure and toxicological properties has arisen in order to evaluate the human health hazards discussed in the CLH dossier.

As mentioned above, the DS proposed read-across from BMP. RAC, following suggestion from a commenting Member State Competent Authorities (MSCA) during the general consultation, 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.

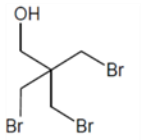
2,3-DBPA is mentioned in the CLH dossier as member of the Small Brominated linear and branched Alkyl Alcohols (SBAA) group described by the Danish Environment Protection Agency (DEPA) in its respective report entitled "Category approach for selected brominated flame retardants - preliminary structural 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

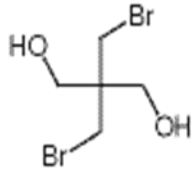


Dominant chemical structure of the SBAA group

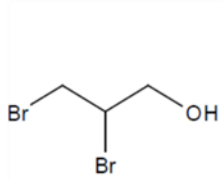
The most prominent members of the SBAA group



2,2-dimethylpropan-1-ol, tribromo derivative; 3-bromo-2,2-bis(bromomethyl)propan-1-ol (TBNPA)
EC 253-057-0
CAS 36483-57-5, 1522-92-5



2,2- bis(bromomethyl)propane-1,3-diol (BMP)
CAS 3296-90-0



2,3-Dibromo-1-propanol (2,3-DBPA)
CAS 96-13-9

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 nuclear 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, while 2,3-DBPA (which has harmonised classification as Carc. 1B and Repr. 2) did not result in the same clusters . 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 physico-chemical and structural properties for TBNPA, BMP and 2,3-DBPA that are of interest in the present opinion are summarised in the following table. The physico-chemical properties between these 3 SBAAs present similarities and differences, with some properties of 2,3-DBPA lying between the values reported for TBNPA and BMP (relative density, LogP, 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 physicochemical properties of these three SBAAs.

***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 colourless 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 internal reabsorption, or whether BMP or its glucuronide metabolite is the active compound. On the other hand, similar to many halogenated aliphatic alcohols, 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.

A summary of the available experimental data for TBNPA, BMP and 2,3-DBPA (data sources: CLH report for TBNPA; RAC opinion for BMP; Treinen *et al*., 1989; Lamb *et al*., 1997; Lamb *et al*., 1997; NTP, 1993; ECB, 1998) is showm below.

Table: *Summary of all available experimental data for TBNPA, BMP and 2,3-DBPA*

EXCRETION AND METABOLISM			
	TBNPA	**BMP**	**2,3-DBPA**
CAS	36483-57-5; 1522-92-5	3296-90-0	96-13-9
EC	253-057-0	221-967-7	202-480-9
Studies	No studies	1. Hoehle *et al.,* 2009 2. Rad *et al.,* 2010	NTP, 1993
Evaluation	-	Toxicokinetic data on BMP concentrations in the gonads was given in the publication of Hoehle *et al.*, 2009. In the testis of rats only 0.01% BMP was recovered after up to 10 days of exposure. No female rats were used in study. There were no other toxicokinetic data examining whether BMP reaches the ovaries of mammals. No data were available on the distribution of the metabolite(s) before and after internal reabsorption, or whether BMP or its glucuronide metabolite is the active compound. Results of an *in vivo* study with male F-344 rats showed that BMP is extensively excreted in the urine (80% in 12h) solely as a monoglucuronide conjugate (Hoehle *et al.*, 2009). This BMP glucuronide is most likely formed in the liver because it is secreted into the bile at early time points after administration	2,3-DBPA is a metabolite of the flame retardant tris(2,3-dibromopropyi)-phosphate. 2,3-DBPA is structurally analogous to 1,2-dibromo-3-chloropropane, 1,2-dichloropropane and 1,3-dichloropropene. 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.
CARCINOGENICITY STUDIES			
Studies	No studies	NTP (rats and mice, oral) POSITIVE Industry (rats, oral) POSITIVE	NTP (rats and mice, dermal) POSITIVE

Evaluation	Subject of the present ODD	Carc. 1B	Carc. 1B
MUTAGENICITY STUDIES			
Studies	*In vitro* 1. OECD TG 473 Chromosome aberration in mammalian cells, peripheral human lymphocytes, POSITIVE 2. OECD TG 476 Mammalian cell gene mutation assay (mouse lymphoma L5178Y cells, gene mutation), POSITIVE. 3. OECD TG 471 Bacterial reverse mutation assay: *In vitro* gene mutation study in bacteria (Ames test), POSITIVE	*In vitro* 1. OECD TG 471 Bacterial Reverse Mutation Assay, POSITIVE 2. OECD TG 471 Bacterial Reverse Mutation Assay, POSITIVE 3. Similar to OECD TG 471 Bacterial reverse mutation assay, POSITIVE 4. Similar to OECD TG 471 Bacterial reverse mutation assay, NEGATIVE 5. Similar to OECD TG 473 *In vitro* mammalian chromosomal aberration test, POSITIVE 6. Similar to OECD TG 479 Sister chromatid exchange assay in mammalian cells, EQUIVOCAL 7. *In vitro* comet assay BMP induced DNA breaks and oxidative stress tests, POSITIVE	*In vitro* 1. *Salmonella typhimurium* gene mutations, POSITIVE 2. L5178Y mouse lymphoma gene mutations, POSITIVE 3. Sister chromatid exchange assay in Chinese hamster ovary cells *in vitro*, POSITIVE 4. Chromosomal aberrations Chinese hamster ovary cells *in vitro*, POSITIVE
	In vivo 1. OECD TG 486 Unscheduled DNA Synthesis (UDS) test with rat liver cells, NEGATIVE 2. OECD TG 474 *In vivo* mammalian somatic cell study: cytogenicity /erythrocyte micronucleus, NEGATIVE	*In vivo* 1. OECD TG 474 Mouse peripheral blood micronucleus test, POSITIVE 2. OECD TG 474 Mouse bone marrow micronucleus test, POSITIVE 3. OECD TG 489 Mammalian alkaline comet assay, POSITIVE	*In vivo* 1. Sex-linked recessive lethal mutations, Drosophila melanogaster, POSITIVE 2. Reciprocal translations, *Drosophila melanogaster*, POSITIVE 3. Micronucleated erythrocytes, Mouse bone marrow cells, NEGATIVE
Evaluation	Subject of the present ODD	RAC Opinion as Muta. 1B	Evaluated by NTP and the Technical Committee for Classification and Labelling as non-genotoxic, since there are only positive *in vitro* tests and one negative *in vivo*
REPRODUCTIVE TOXICITY - SEXUAL FUNCTION & FERTILITY			
Studies	28-day oral RDT study in rats with a 14 day recovery period where some relevant reproduction parameters were investigated (Anonymous, 2015; REACH registration dossier) NO EFFECTS OBSERVED	NTP, mice, oral using the RACB protocol (Treinen *et al.*, 1989) EFFECTS OBSERVED	➢ Within the NTP Carc study (rats and mice, dermal), there were fertility parameters monitored for 13-weeks EFFECTS OBSERVED ➢ Dermal application of tris(2,3-dibromo-propyl) phosphate which metabolizes to 2,3-DBPA in rabbits (Osterberg *et al.*, 1977) EFFECTS OBSERVED ➢ Intraperitoneal injection of tris(2,3-dibromo-propyl) phosphate which metabolizes to

			2,3-DBPA in rats (Cochran and Wiedow, 1986) EFFECTS OBSERVED
Evaluation	Subject of the present ODD	Not evaluated in the adopted RAC opinion. NTP concluded that BMP is not a selective reproductive toxicant, as the effects observed were concomitant with the general toxicity.	Harmonised classification as Repr. 2, H361f (CLP 0.0) NTP would also classify based on effects on testes and epididymides (decrease in organ weights, reduced sperm density, an increase in abnormal sperm). Comparable findings also occurred in studies on the rat (i.p. administration) and the rabbit (dermal application) involving the structurally analogous substance tris(2,3-dibromopropyl)phosphate from which, *inter alia*, 2,3-DBPA is formed in metabolism
REPRODUCTIVE TOXICITY - DEVELOPMENTAL TOXICITY			
Studies	OECD TG 414 prenatal developmental toxicity study, rats NO EFFECTS OBSERVED	NTP, mice, oral using the RACB protocol (Treinen *et al*., 1989) (same study as in sexual function & fertility) VERY LIMITED EFFECTS OBSERVED	NTP, Two teratogenicity studies on the rat with application by gavage are available for the structurally analogous substance tris(2,3-dibromopropyl)phosphate from which, *inter alia*, 2,3-dibromo-1 -propanol is formed in metabolism VERY LIMITED EFFECTS OBSERVED
Evaluation	Subject of the present ODD	Not evaluated in the adopted RAC opinion. NTP concluded that BMP is not a selective reproductive toxicant, as the effects observed were concomitant with the general toxicity. Any effects observed, though, were not developmental observations.	NTP concluded that studies on the rat involving the structurally analogous substance tris(2,3-dibromopropyl)phosphate did not provide any indications of a teratogenic effect. Foetal development was only retarded in the highly maternal-toxic range.
SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE			
Studies	1. OECD TG 407, GLP, 28-day oral study in rats, SLIGHT REVERSIBLE EFFECTS (liver, kidney) 2. Non guideline, oral by gavage 14-day, rats, NEGATIVE (stained urine) 3. Non guideline, 30-day feeding study, rats SLIGHT REVERSIBLE EFFECTS (kidney, urine bladder) 4. OECD TG 408, 90-day oral study in	Elwell *et al.*, 1989 (NTP 1996, TR-452) Range finding 13-week oral study in rats and mice, large doses, not many effects. In rats no chemical-related clinical findings, in clinical pathology increased urine volumes accompanied by decreased urine specific gravity and minimally increased protein excretion, kidney and liver are the target tissues, Renal papillary degeneration was present males, and in 20000 ppm males and females. Hyperplasia of the urinary bladder was present in 20000 ppm males. In mice, clinical findings included abnormal posture and hypoactivity in 10000 ppm male and female mice, increased blood urea nitrogen concentrations in males and	Eustis *et al*., 1994 (NTP, 1993) Range finding 13-week oral study in rats and mice, large doses, not many effects. In rats, chemical-related lesions occurred in the kidney of male rats and in the liver of female rats. In mice chemical related lesions occurred in the liver and lung.

	rats SLIGHT REVERSIBLE EFFECTS (kidney, urine bladder)	females, papillary necrosis, renal tubule regeneration, and fibrosis were observed in the kidneys of 2500 and 5000 ppm males and 10000 ppm males and females. Urinary bladder hyperplasia was observed in 5000 and 10000 ppm males and females.	
Evaluation	Subject of the present ODD	Not previously evaluated	Not previously evaluated

Based on the available studies, the toxicological properties for the three SBAAs can be summarised as follow.

- 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 serum glutamic-pyruvic transaminase [SGPT] activity, increased liver weight and minimal centrilobular hypertrophy) 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, Thymidine Kinase 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) but with limitations, while BMP was positive in two different *in vivo* erythrocyte micronucleus 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 as shown in the tables below:

Table: *Common tumours in rats for BMP and DBPA*

Site	BMP	DPBA	BMP	DPBA
	Rats		Rats	
	Male	Male	Female	Female
Skin	+	+		
Zymbal's gland	+	+		
Mammary gland			+	+
Oral cavity - Oral Mucosa	+	+	+	+
Oesophagus	+	+	+	+
Forestomach	+	+		
Small intestine	+	+		
Large intestine	+	+		
Kidney	?	+		

Table: *Common tumours in mice for BMP and DBPA*

Site	BMP	DBPA	BMP	DBPA
	Mice		Mice	
	Male	Male	Female	Female
Forestomach	+	+	?	+
Lung	+	+	+	?

+: positive results
?: equivocal results

RAC has applied the Read-Across Assessment Framework (RAAF) (2017) developed by ECHA for the two possible source substances, BMP and 2,3-DBPA, and the results are shown below.

RAAF SCENARIO 2 – TBNPA/BMP

This scenario covers the analogue approach for which the read-across hypothesis is based on different compounds which have the same type of effect(s).

ASSESSMENT ELEMENT	PARAMETERS - ASSESSMNET	SCORE
AE A.1 CHARACTERISATION OF SOURCE SUBSTANCE	Is the substance characterization including the impurity profile provided for the source substance? **BMP 80% purity from the NTP 1996, TR-452 (CLH-O-0000001412-86-212/F)**	4
AE A.2 LINK OF STRUCTURAL SIMILARITY AND DIFFERENCES WITH THE PROPOSED PREDICTION	• the scientific hypothesis establishes the structural similarities and differences of source and target; • structural similarities and differences are linked with the possibility to predict similar properties; and • the provided evidence supports the proposed link between structural similarities and the possibility to predict. **Structural similarity, chemical reactivity well established.** **In the same biological environment TBNPA and BMP are expected to behave similarly.**	5
AE A.3 RELIABILITY AND ADEQUACY OF THE SOURCE STUDY	• The study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across • The test material used represents the source substance as described in the hypothesis in terms of purity and impurities. **NTP 1996, TR-452 (CLH-O-0000001412-86-212/F)**	5
AE A.4 Bias	TBNPA and BMP are in the same (Q)SAR-based clusters for carcinogenicity and genotoxicity **Danish EPA Report**	5
AE 2.1 COMPOUNDS THE TEST ORGANISM IS EXPOSED TO	• the compounds to which the test organism is exposed (after administration of the source and the target substances) have been established in the documentation; and • the provided evidence supports the explanation. **TBNPA >97% purity – no toxicokinetics/ metabolism, impurities in the Registration dossier unknown 0.1-1.34%, 3 well- characterised (e.g. 0.9% BMP)** **For BMP NTP 1996, TR-452 (Hoehle et al., 2009) (CLH-O-0000001412-86-212/F)**	4
AE 2.2 COMMON UNDERLYING MECHANISM, QUALITATIVE ASPECTS	• the documentation has established a common underlying mechanism; • this mechanism links the structures of the compounds under consideration with the possibility to predict qualitatively similar type of effects for the target substance for the property under consideration; and	4
	• the provided evidence support the explanation. **Experimental MoA/MoAs not established. Chemical structure/ reactivity established.** **Regardless of how many biological MoAs are operating, in the same biological environment TBNPA and BMP are expected to behave similarly.**	
AE 2.3 FORMATION AND IMPACT OF NON-COMMON COMPOUNDS	Does the common underlying mechanism quantitatively* link the compounds to which the organism is exposed to the prediction for the property under consideration? **Experimental MoA/MoAs not established.** **Regardless of how many biological MoAs are operating, in the same biological environment TBNPA and BMP are expected to behave similarly.**	3
AE 2.4 EXPOSURE TO OTHER COMPOUNDS THAN TO THOSE LINKED TO THE PREDICTION	Are there indications that other compounds than those linked to the prediction may be formed or may be present as impurities? **TBNPA >97% purity – no toxicokinetics/ metabolism, 2 constituents present (1,2) with no toxicokinetics/ metabolism /toxicological data** **Experimentally, the toxicokinetic data for TBNPA and BMP is very limited, as the only known metabolite is the glucuronidation product of BMP. Glucuronidation in general represents a common metabolic pathway of xenobiotics in order to render them more hydrophilic and facilitate renal excretion. This pathway could not solely account for all toxicological effects observed.**	4
AE 2.5 OCCURRENCE OF OTHER EFFECTS THAN COVERED BY THE HYPOTHESIS AND JUSTIFICATION	It has to be assessed whether: • additional mechanisms than those identified in the hypothesis may be acting: ✓ on the basis of mechanistic insights; or ✓ derived from information in the data matrix. • these additional mechanisms affect the prediction for the property under consideration. **Experimental MoA/MoAs not established. Chemical structure/ reactivity established.** **Regardless of how many biological MoAs are operating, in the same biological environment TBNPA and BMP are expected to behave similarly.**	4

RAAF SCENARIO 2 – TBNPA/2,3-DBPA

This scenario covers the analogue approach for which the read-across hypothesis is based on different compounds which have the same type of effect(s).

ASSESSMENT ELEMENT	PARAMETERS - ASSESSMNET	SCORE
AE A.1 CHARACTERISATION OF SOURCE SUBSTANCE	Is the substance characterization including the impurity profile provided for the source substance? **NTP 1993, TR-400, PURITY ≥ 98%**	5
AE A.2 LINK OF STRUCTURAL SIMILARITY AND DIFFERENCES WITH THE PROPOSED PREDICTION	• the scientific hypothesis establishes the structural similarities and differences of source and target; • structural similarities and differences are linked with the possibility to predict similar properties; and • the provided evidence supports the proposed link between structural similarities and the possibility to predict. **Structural and chemical reactivity similarity with deficiencies.**	3
AE A.3 RELIABILITY AND ADEQUACY OF THE SOURCE STUDY	• The study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across • The test material used represents the source substance as described in the hypothesis in terms of purity and impurities. **NTP 1993, TR-400**	5
AE A.4 Bias	TBNPA and 2,3-DBPA are in the same structural group (10) of BFRs but in different clusters **Danish EPA Report**	4
AE 2.1 COMPOUNDS THE TEST ORGANISM IS EXPOSED TO	• the compounds to which the test organism is exposed (after administration of the source and the target substances) have been established in the documentation; and • the provided evidence supports the explanation. **TBNPA >97% purity – no toxicokinetics/ metabolism, 2 constituents present (1,2) with no toxicokinetics/ metabolism /toxicological data** **For 2,3-DBPA NTP 1993, TR-400 purity is ≥ 98%, experimentally dehydroalogenation (-HBr) eventually producing 2-bromoacrylic acid (detected as a metabolite) is one metabolic pathway. In addition, 2,3-DBPA may undergo oxidation to form epoxides, which can enter different metabolic pathways. These 2 unique metabolic routes for 2,3-DBPA, probably do not operate in the other 2 SBAAs (BFRs). On the other hand, all the mechanisms described above for TBNPA and BMP cannot be excluded for 2,3-DBPA.**	3
AE 2.2 COMMON UNDERLYING MECHANISM, QUALITATIVE ASPECTS	• the documentation has established a common underlying mechanism; • this mechanism links the structures of the compounds under consideration with the possibility to predict qualitatively similar type of effects for the target substance for the property under consideration; and • the provided evidence support the explanation. **The structures, chemical reactivity, MoAs and toxicological properties share similarities but with substabtial deficiencies.**	3
AE 2.3 FORMATION AND IMPACT OF NON-COMMON COMPOUNDS	Does the common underlying mechanism quantitatively* link the compounds to which the organism is exposed to the prediction for the property under consideration? **The structures, chemical reactivity, MoAs and toxicological properties share similarities but with substabtial deficiencies.**	3
AE 2.4 EXPOSURE TO OTHER COMPOUNDS THAN TO THOSE LINKED TO THE PREDICTION	Are there indications that other compounds than those linked to the prediction may be formed or may be present as impurities? **TBNPA >97% purity – no toxicokinetics/ metabolism, 2 constituents present (1,2) with no toxicokinetics/ metabolism /toxicological data** **For 2,3-DBPA NTP 1993, TR-400 purity is ≥ 98%, experimentally dehydroalogenation (-HBr) eventually producing 2-bromoacrylic acid (detected as a metabolite) is one metabolic pathway. In addition, 2,3-DBPA may undergo oxidation to form epoxides, which can enter different metabolic pathways. These 2 unique metabolic routes for 2,3-DBPA, probably do not operate in the other 2 SBAAs (BFRs). On the other hand, all the mechanisms described above for TBNPA and BMP cannot be excluded for 2,3-DBPA.**	3
AE 2.5 OCCURRENCE OF OTHER EFFECTS THAN COVERED BY THE HYPOTHESIS AND JUSTIFICATION	It has to be assessed whether: • additional mechanisms than those identified in the hypothesis may be acting: ✓ on the basis of mechanistic insights; or ✓ derived from information in the data matrix. • these additional mechanisms affect the prediction for the property under consideration. **For 2,3-DBPA NTP 1993, TR-400 purity is ≥ 98%, experimentally dehydrohalogenation (-HBr) eventually producing 2-bromoacrylic acid (detected as a metabolite) is one metabolic pathway. In addition, 2,3-DBPA may undergo oxidation to form epoxides, which can enter different metabolic pathways. These 2 unique metabolic routes for 2,3-DBPA, probably do not operate in the other 2 SBAAs (BFRs). On the other hand, all the mechanisms described above for TBNPA and BMP cannot be excluded for 2,3-DBPA.**	3

It is evident that RAAF indicates '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'. This is in line with the partial similarity of 2,3-DBPA with TBNPA, substantiated above, with regards to chemical structure and reactivity, physico-chemical 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, data is read-across from BMP (but not 2,3-DBPA) for the reasons stated above.

5 IDENTIFIED USES

According to the information published on the ECHA dissemination site, this substance is used in the manufacture of polymers, plastic products and chemicals and as an intermediate.

TBNPA is used as a reactive flame retardant in polymers synthesis (100-1000 tpa) for the manufacture of plastic products and chemicals. It is used in industrial, professional and consumer settings in formulation and use of commercial mixture(s).

6 DATA SOURCES

- Report: Category approach for selected brominated flame retardants (Wedebye et al., 2016)
- RAC-opinion, CLH-report/annex and Risk Management Option Analysis for similar substance 2,2-bis(bromomethyl)propane-1,3-diol (BMP), CAS no. 3296-90-0
- REACH registration via ECHA´s dissemination site
- CSR in the REACH registration via ECHA´s Remote access portal/IUCLID
- Internet resources:

  eChemPortal

  Toxnet/Toxline/Pubmed/PubChem

  Search engine Google –www.google.com

Date of search: The period from initiation of the work until submission (large parts of 2018).

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties of TBNPA

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid, white to off-white flakes	ECHA[1]	
Melting/freezing point	Melting / freezing point at 101 325 Pa: 68.96°C	"	
Boiling point	N.A.[2]	"	
Relative density	Relative density at	"	

[1] ECHA dissemination site

[2] N.A. Not available

Property	Value	Reference	Comment (e.g. measured or estimated)
	20C:2.286		
Vapour pressure	0 Pa at 25°C	"	
Surface tension	N.A.	"	
Water solubility	1.93 g/L at 20.1°C	"	
Partition coefficient n-octanol/water	Log Kow (Log Pow):2.6 at 22.5°C	"	
Flash point	N.A.	"	
Flammability	N.A.	"	
Explosive properties	N.A.	"	
Self-ignition temperature	N.A.	"	
Oxidising properties	N.A.	"	
Granulometry	N.A.	"	
Stability in organic solvents and identity of relevant degradation products	No change was found in the concentration of 2,2-dimethylpropan-1-ol, tribromo derivative (TBNPA) over 14 days, within the method's precision capability.	"	
Dissociation constant	N.A.		
Viscosity	N.A.		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No data available

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity/Skin corrosion/Irritation/Eye damage/Sensitisation

Not evaluated in this dossier.

10.1 Germ cell mutagenicity

Table 3: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro cytogenicity / chromosome aberration study in mammalian cells (lymphocytes: Peripheral human lymphocytes) OECD TG 473	TBNPA (CAS no. 36483-57-5) Purity 97%	Based on range finding study, the doses in the main studies ranged from 100 to 2000 µg/ml with and without metabolic activation (S-9 mix). Positive control was MMC, Negative control (solvent only) was DMSO 3hr exposure, 24 hr fixation.	**TBNPA was found to be clastogenic** in the presence of metabolic activation, and at the highest test substance concentration (1000 microgram/ml) in the absence of metabolic activation. Cytotoxicity seen as low as at 100 µg/ml, with metabolic activation. TBNPA has the **potential to disturb mitotic processes and cell cycle progression,**	Study report unnamed, 2004
Mammalian cell gene mutation assay (Mouse lymphoma L5178Y cells, gene mutation) following OECD TG 476	TBNPA (CAS no. 36483-57-5) Purity 97%	Following a range finding test, in the main studies, concentrations varied from 10 to 535 µg/ml, and up to 8 dose groups pluss two solvent controls, e.g. 10, 50, 100, 200, 300, 400, 500 µg/ml in experiment 1 (without metabolic activation). Positive control methyl methane sulfonate/cyclophosphamide	TBNPA was **mutagenic** in the test system with incubations in the presence of metabolic activation (S9-mix). Cytotoxicity was seen atconcentration of 333 µg/plate and above	Study report unnamed, 2004
Bacterial reverse mutation assay: In vitro gene mutation study in bacteria (Ames test) OECD TG 471	TBNPA Purity 98%	The Ames test was done with S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (with and without metabolic activation from rodent S-9 mix). Following a preliminary test with 5000 µg/plate, the maximum concentration was 1500 µg/plate in the main test.	Cytotoxicity was seen at 5000 µg/plate. **Mutagenicity was seen** in both mutation tests with strains TA 1535 and TA 100 at concentrations between 15 and 500 µg/plate, but only with metabolic activation.	Study report unnamed, 1996

Table 4: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Unscheduled DNA Synthesis (UDS) test with rat liver cells (hepatocytes) *in vivo*. GLP and OECD TG 486	TBNPA Purity not given	Sprague-Dawley rats (CD (Ctr;CD (SD) IGS BR) strain) *No. of animals per sex per dose:* 2 range finder studies: (1 male; 1 female) and (2 males 0 females); 2 main tests: study 1: 4 per dose (males); study 2: 4 per dose (males) All animals were dosed once. In the range finding tests the dose was 2000 mg/kg. In the main studies the dose was 670 and 2000 mg/kg bw. Administration: . oral by gavage. Treatment: 16 hr (experiment 1); 2 hr (experiment 2). Negative control: number of animals not given Positive control 2-Acetamididofluorene (2AAF) at 50 mg/kg bw, and Sym-Dimethylhydrazine dihydrochloride (NDHC) at 40 mg/kg bw.	Negative result: The test material did not induce any marked or toxcologiacally significant increases in the incidence of cells undergoing DNA synthesisin isolated rat hepatocytes following in vivo exposure for 2 or 16 hr. Therefore **the test material was considered to be non-genotoxic under the conditions of the study.** Concurrent positive control data: Both positive controls produced marked increases in the incidence of cells in repair and the vehicle control groups gave **acceptable values for net nuclear grain counts.** Administration of the test substance in the range finding study produced **toxicity in the dosed animals manifested as ataxia, lethargy, red colored urine** (no deaths). Lethargy and ataxia was also seen in the main studies.	Study report unnamed (2007a)
In vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus OECD test guideline 474 is relevant, but the study was done prior to the guideline. No major deviations from the guideline. Reliability score made by the	TBNPA Degree of purity: 98.1%	NMRI mice / male and female No. of animals per sex per dose: In total 81 animals (45 males and 36 females). Ten animals (5 males, 5 females) per dose. Preliminary test: 2000, 1500, 1000, 500,400, 300 (mg/kg b.w) main test: 300, 150, 75 (mg/kg bw. On the day of the experiment, the test item was formulated in DMSO+corn oil (30%-70%). The vehicle was chosen to its relative non-toxicity for the animals. All animals received a single standard volume of 10 mL/kg body weight orally. Negative controls: "valid"	**TBNPA did not induce micronuclei as determined by this micronucleus test** with femur bone marrow cells of the mouse. The % micronuclei was 0.085, 0.110 and 0.125 at dose 75, 150, 300 mg/kg bw 24 hours post-treatment.	Study report unnamed (2007b)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
registrant: 1		(no more information available) Positive control substance(s): CPA; Cyclophosphamide (>98%); Dosing: 40 mg/kg b.w ; volume administration: 10 mL/kg b.w		

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

In vitro studies:
In the OECD TG 473 *In vitro* cytogenicity / chromosome aberration study in mammalian cells TBNPA was found to be clastogenic in the presence of metabolic activation and at the highest test substance concentration (1000 microgram/ml) in the absence of metabolic activation. TBNPA has the potential to disturb mitotic processes and cell cycle progression (Study report unnamed, 2004).

The OECD TG 476 Mammalian cell gene mutation assay was positive. TBNPA was mutagenic in the test system with incubations in the presence of metabolic activation. The presence of S9-mix in both tests resulted an increase in mutation frequencies more than threefold and outside the labs historical data (no more detailed information about historical data is available in the registration). The increases were considered biologically relevant and TBNPA is considered mutagenic in vitro (Study report unnamed, 2004).

In the *in vitro* assays one Ames test was included. In the presence of hamster S-9 mix however, there were clear evidence of mutagenic activity between 500 and 15 µg/plate with strains TA 1535 and TA 100. The test showed no evidence of mutagenic activity in the absence or presence of rat S-9 mix (Study report unnamed, 1996).

In vivo studies: In the *in vivo* mammalian somatic cell study TBNPA did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore TBNPA can be considered to be non-mutagenic in this test (Study report, unnamed, 2007b).

In the OECD TG 486 Unscheduled DNA Synthesis (UDS) test with rat liver cells (liver hepatocytes) *in vivo* TBNPA did not induce any marked or toxicologically significant increases in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat hepatocytes following in vivo exposure for 2 or 16 hr. Therefore, the test material was considered to be non-genotoxic under the conditions of the study (Study report, 2007a).

10.1.2 Other relevant information

TBNPA belongs to a small category of brominated substances and an analogue read-across approach is proposed for mutagenicity and carcinogenicity, see section 10.2.1 below.

10.1.3 Comparison with the CLP criteria for mutagenicity

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

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

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

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

Category 2: Classification criteria for category 2, from CLP: "Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: – Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: – Somatic cell mutagenicity tests in vivo, in mammals; or – Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays"

Rationale for proposal for classification in Cat. 1B:

TBNPA was clastogenic in human lymphocytes in vitro in the presence of metabolic activation and at the highest test concentration without metabolic activation, and mutagenic in mouse lymphoma cells in vitro in the presence of metabolic activation. In bacterial reverse mutation assays, mutagenicity was seen. 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. We have no reproductive toxicity studies that indicate that TBNPA reaches the germ cells. The database is limited to a single prenatal developmental toxicity study. However, as described above, we propose to read across from the source substance BMP to the target substance TBNPA, see section 10.2.1 and table 10 (Data matrix for studies relevant for assessing germ cell mutagenicity, Analogue Approach). RAC states in the recent RAC-opinion for BMP that "there is positive evidence of somatic cell mutagenicity for BMP from in vitro/in vivo studies and evidence from the reproductive toxicity studies and that this supports that BMP reaches the (female) germ cells". According to RAC "both facts in combination are sufficient to give 'some' evidence that the substance has the potential to cause mutations to germ cells". RAC agreed that BMP should be classified as a germ cell mutagen, Cat. 1B; H340. We propose the same classification for TBNPA.

10.1.4 Conclusion on classification and labelling for mutagenicity

TBNPA should be classified as Muta. 1B, H340

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS based the evaluation of mutagenic properties of TBNPA on the following studies:

1. *In vitro studies:*

i) In the OECD TG 473 *in vitro* cytogenicity/chromosome aberration study in mammalian cells, TBNPA was found to be clastogenic in the presence of metabolic activation and at the highest test substance concentration (1000 µg/mL) in the absence of metabolic activation. TBNPA has the potential to disturb mitotic processes and cell cycle progression (Anonymous, 2004a).

ii) The OECD TG 476 mammalian cell gene mutation assay was positive. TBNPA was

mutagenic in the test system with incubations in the presence of metabolic activation. The presence of S9-mix in both tests resulted in an increase in mutation frequencies more than threefold and outside the labs historical data (no more detailed information about historical data is available in the registration). The increases were considered biologically relevant and TBNPA is considered mutagenic *in vitro* (Anonymous, 2004b).

iii) In the OECD TG 471 Ames test, in the presence of hamster S9-mix, there was clear evidence of mutagenic activity between 500 and 15 µg/plate with strains TA1535 and TA 100. The test showed no evidence of mutagenic activity in the absence or presence of rat S9-mix (Anonymous, 1996).

2. In vivo studies:

i) In the *in vivo* mammalian somatic cell study (similar to OECD TG 474, no major deviations), TBNPA did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore TBNPA can be considered to be non-mutagenic in this test (Anonymous, 2007b).

ii) In the OECD TG 486 UDS test with rat liver cells (liver hepatocytes) *in vivo* TBNPA did not induce any marked or toxicologically significant increases in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat hepatocytes following *in vivo* exposure for 2 or 16h. Therefore, the test material was considered to be non-genotoxic under the conditions of the study (Anonymous, 2007a).

The DS concluded that TBNPA was clastogenic in human lymphocytes *in vitro* in the presence of metabolic activation and at the highest test concentration without metabolic activation, and mutagenic in mouse lymphoma cells *in vitro* in the presence of metabolic activation. In bacterial reverse mutation assays, mutagenicity was also seen. 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. The DS added that there were no suitable studies to indicate that TBNPA reaches the germ cells. The database available for TBNPA is limited to a single prenatal developmental toxicity study.

The DS, recognizing the limited database on TBNPA available for reaching a conclusion, proposed to read-across from the substance BMP, which was evaluated by RAC (adopted RAC opinion CLH-O-0000001412-86-212/F, 8 June 2018) and classified as a germ cell mutagen 1B. Originally, the DS in the CLH dossier proposed the same classification (germ cell mutagen 1B; H340) for TBNPA. After receiving comments both from Industry and from MSCAs during the consultation (for more details see section below), the DS recognized that:

- blood plasma results provided by Industry showed bioavailability for TBNPA
- the sensitivity of different bacterial strains used for the *in vitro* testing and whether 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). Nevertheless, the DS still considered the results of the Ames test to be positive in both mutation tests, but only in the highest concentration for TA100.
- the results on the germ cells in the 28-day toxicity study show that no treatment related changes in sperm count and motility were observed.
- the shortcomings in the *in vivo* data, such as the dose selection in the *in vivo* micronucleus test or the general validity of the UDS test.

The DS, after reconsidering the available information listed above, agreed that the note to table 3.5.1 in CLP section 3.5.2.2 can be considered relevant to TBNPA based on the QSAR clustering for genotoxicity (BMP and TBNPA in same cluster; Wedebye *et al*., 2016), and thus revised the original conclusion and proposed TBNPA to be classified as a germ cell mutagen, Category 2.

Comments received during consultation

There were 4 comments received during the consultation, 1 from Industry and 3 from MSCAs.

Industry argued that the proposed hazard category for germ cell mutagen 1B is too severe based on the TBNPA database. While Industry recognized that TBNPA and BMP share similarities in the *in vitro* mutagenicity assays, the results in the *in vivo* tests are substantially different. Since the available experimental data for TBNPA are sufficiently robust and indicates that the substance is not an *in vivo* genotoxin, Industry considered that there is no need to read-across from BMP. Therefore, they suggested classification by the DS should be removed based on the lack of *in vivo* genetic damage shown experimentally.

The 3 MSCAs did not in general reject the category approach established by the Danish Environmental Protection Agency and applied by the DS within the current proposal, but expressed concerns regarding the category proposed by the DS for classification for germ cell mutagenicity. All 3 rather favoured classification in category 2, rather than 1B. The MSCAs supported the read-across from BMP, supporting a classification in Category 2. Two of the 3 commenting MSCAs provided similar reasoning and arguments with regards to shortcomings of the *in vivo* data set for TBNPA, which were accepted by the DS and led to review of the classification proposed for TBNPA to germ cell mutagen Cat. 2).

Assessment and comparison with the classification criteria

The following tables present a summary of the mutagenicity/genotoxicity tests *in vitro* and *in vivo* for TBNPA:

***Table**: Summary of mutagenicity/genotoxicity tests in vitro*

Study/Method/ Guideline/deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**
Study report Anonymous, 2004a *In vitro* cytogenicity/chromosome aberration study in mammalian cells (lymphocytes: peripheral human lymphocytes) OECD TG 473, GLP	TBNPA (commercial preparation FR-513) Purity 97%	Based on range finding study, the doses in the main studies ranged from 100 to 2000 µg/mL with and without metabolic activation (S9-mix**). √ Positive control was Mitomycin C (MMC), a clastogen active without metabolic activation, and cyclophosphamide (CP), a clastogen requiring metabolic activation	TBNPA **was found to be clastogenic** in the presence of metabolic activation (Aroclor-1254 induced rat liver S9-mix, according to the Industry the activation was a 1.8% in culture media S9-mix fraction of Aroclor induced rat liver homogenates), and at the highest test substance concentration

		√ Negative control (solvent only) was DMSO 3h exposure, 24 h fixation. Vehicle choice according to TG 473	(1000 µg/mL) in the absence of metabolic activation. Cytotoxicity seen as low as at 100 µg/mL, with metabolic activation. TBNPA has the **potential to disturb mitotic processes and cell cycle progression (chromatid and chromosome breaks, some minutes, single usually circular, part of a chromatid lacking a centromere, polyploidy).**
Study report Anonymous, 2004b Mammalian cell gene mutation assay (mouse lymphoma L5178Y cells, gene mutation) following OECD TG 476, GLP compliant	TBNPA (commercial preparation FR-513) Purity 97%	Dose range finding test (without/with metabolic activation): Solvent control, 33, 100, 333, 1000, 3250 µg/mL Metabolic activation: rat liver microsomal enzymes, S9-fraction** Experiment 1 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 200, 300, 400, 500, positive control (MMC) Experiment 1 (with metabolic activation 8%): first solvent control, second solvent control, 50, 100, 200, 300, 375, 450, 500, positive control (CP) Experiment 2 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 225, 250, 300, 325, 350, positive control (MMC) Experiment 2 (with metabolic activation 12%): first solvent control, second solvent control, 100, 200, 300, 350, 400, 470, 500, 535, positive control (CP) Positive control methyl methane sulfonate/cyclophosphamide Vehicle DMSO	Mutant frequencies: In the absence of S9-mix TBNPA did not induce a significant increase in mutant frequencies in the first experiment. This result was confirmed in a repeat experiment with modifications in the duration of the time treatment from 3 to 24 hours. FR-513 was mutagenic in the test system with incubations in the presence of metabolic activation. The presence of S9-mix in both tests resulted in an increase in mutation frequencies colonies more than threefold and outside the laboratoty historical data (exact data not available). Industry claimed a 6.6-fold increase in the mutant frequency at the TK locus and up to an 8-fold increase in small colonies and a 5.4-fold increase in large colonies. The second experiment confirmed the positive result for both small and large.

			The increases were considered biologically relevant and FR-513 is considered mutagenic *in vitro*. Cytotoxicity was seen at concentration of 333 µg/plate and above with and without metabolic activation.
Study report Anonymous, 1996 Bacterial reverse mutation assay: *in vitro* gene mutation study in bacteria (Ames test) OECD TG 471	TBNPA (commercia preparation FR-513) Purity 98%	*S. typhimurium* TA1535, TA1537, TA 98 and TA 100# (with and without metabolic activation from hamster S9-mix treated with Aroclor-1254**). Strains TA98 and TA1537 are capable of detecting frameshift mutagens, strains TA100 and TA1535 are capable of detecting base-pair substitution mutagens. Test concentrations: 0, 5, 50, 500, 5000 µg/plate in the preliminary toxicity determination (with and without metabolic activation), and 0, 15, 50, 150, 500, 1500 µg/plate in the main test (with and without metabolic activation).	5000 µg/plate in the preliminary toxicity determination was toxic so the highest concentration was set to 1500 µg/plate in the main test. Toxicity was observed in the preliminary test at the concentration of 5000 µg/plate. Large, dose-related increases* in revertant colony numbers were observed in both mutation tests with strains TA1535 and TA100, at concentrations between 15 and 500 µg/plate, but this was only observed with metabolic activation. DMSO used as negative control. Positive control was Congo red (CAS no. 573-58-0) which demonstrated the sensitivity of the assay and the metabolizing activity of the liver preparations. **Mutagenicity** was reported in both mutation tests with strains TA1535 and TA100 at concentrations between 15 and 500 µg/plate, only with metabolic activation.

** According to Industry comment, at the time of the test, the laboratory used evaluation criteria that judged a positive response, when reproducible increases in revertants were at least 1.5 times the concurrent solvent controls. Since that time, the current evaluation criteria have changed. For TA1535 and TA1537 a positive response requires a 3-fold increase in revertants and for TA98 and TA100 a positive response requires demonstration of a 2-fold induction of revertants. Based on these criteria, the response in strain TA100 would be assessed as weakly positive in the 30% hamster S9 activation system*

and negative with the 10% hamster S9 activation system. TA1535 had positive responses. Nevertheless, it has been shown that the 2-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).

*** S9 source: Rat liver has a high level of P450 enzymes that will either activate or inactivate promutagens. Aroclor induction increases the concentration of these types of metabolic enzymes in the rat liver. Hamster liver S9 has a different set of metabolic enzymes. The modification using hamster S9 was originally designed to increase the sensitivity of the Ames test to azo dyes and aromatic amines which have traditionally been negative in the Ames test. The hamster S9 enhances the reduction of azo-bonds leading to DNA reactive metabolites. Overall, the rat P450 system enhances oxidation. It must also be remembered that the enzyme systems that activate chemicals can also inactivate them, in particular by glucuronidation more prevalent in the rat liver activation system. Most alkyl halides are metabolically activated by P450 systems (Industry comment).*

 # Strain specificity: Both TA1535 and TA100 carry the same defective histidine gene, hisG46. They both also contain the mutation in the uvrB gene making the strains deficient in DNA repair processes. In order to increase the sensitivity of the tester strains, a plasmid, pKM101, has been inserted in TA1535 to create TA100. This plasmid codes for an error-prone repair process which results in increased sensitivity to mutagens. The presence of the error-prone repair system seemed to mitigate the mutagenicity of FR-513 rather than enhance it. Some lesions could be most likely repaired in TA100, which would explain the different intensity of positive responses in TA1535 and in TA100.

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

Study/Method/ Guideline/deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**
Study report Anonymous, 2007 UD) test with rat liver cells *in vivo.* GLP and OECD TG 486	TBNPA (commercial preparation FR-513) Purity not given	Sprague-Dawley rats (CD (Ctr;CD(SD) IGS BR) strain) No. of animals per sex per dose: 2 range finder studies: (1 male; 1 female) and (2 males 0 females); 2 main tests: study 1: 4 per dose (males); study 2: 4 per dose (males) All animals were dosed once. In the range finding tests the dose was 2000 mg/kg bw. In the main studies the dose was 670 and 2000 mg/kg bw. Administration: oral by gavage. Treatment: 16h (experiment 1); 2h (experiment 2). Negative control:	Results: Negative The test material did not induce any marked or toxicologically significant increases (actual data not available) in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat hepatocytes following *in vivo* exposure for 2 or 16h. Therefore, **the test material was considered to be non-genotoxic under the conditions of the study.** Concurrent positive control data: Both positive and negative controls produced marked increases in the incidence of cells in repair and the vehicle control groups gave **acceptable values for net nuclear grain counts.** Administration of the test substance in the range finding study produced **toxicity in the dosed animals manifested as ataxia, lethargy, red coloured urine** (no deaths). Lethargy and ataxia were also seen in the main studies.

		animals not treated Positive control 2-acetamididofluorene at 50 mg/kg bw, and sym-dimethylhydrazine dihydrochloride at 40 mg/kg bw.	
Study report Anonymous, 2007 *In vivo* mammalian somatic cell study: cytogenicity/ erythrocyte micronucleus OECD TG 474 is relevant, but the study was done prior to the guideline. No major deviations from the guideline.	TBNPA (commercial preparation FR-513) Purity: 98.1%	NMRI mice male and female No. of animals per sex per dose: Ten animals (5 males, 5 females) per dose. Preliminary test: 2000, 1500, 1000, 500, 400, 300 (mg/kg bw/d) main test: 300, 150, 75 (mg/kg bw. On the day of the experiment, the test item was formulated in DMSO+corn oil (30%-70%). The vehicle was chosen to its relative non-toxicity for the animals. All animals received a single standard volume of 10 mL/kg bw orally. Negative controls: "valid" (no more information available) Positive control substance(s):	**TBNPA did not induce micronuclei as determined by this micronucleus test** with femur bone marrow cells of the mouse. The % micronuclei was 0.085, 0.110 and 0.125 at dose 75, 150, 300 mg/kg bw 24 hours post-treatment.

		Cyclophosphamide (CPA > 98%); Dosing: 40 mg/kg bw; volume administration: 10 mL/kg bw	

Based on the data presented above and 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 consultation (cf. public attachment to the RCOM).
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 (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 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/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 notes 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.

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 is high. 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*.

6. 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. Mutagenicity data on BMP are briefly presented and not assessed, as these data come from an adopted RAC opinion for germ cell mutagenicity 1B.

Data on BMP

The database for BMP comprised of three *in vitro* Ames tests, two giving clear concentration related positive results in the presence of 30% Syrian hamster liver S9-mix (National Toxicology, 1996; Zeiger *et al*., 1992) and one with negative result where the S9-mix concentrations were limited to 10% (Mortelmans *et al*., 1986). In summary, positive findings were obtained when using high concentrations of hamster S9-mix (30%), while no mutagenic activity was detected when using rat liver S9-mix, or low concentrations of hamster S9-mix for metabolic activation. Moreover the OECD TG 473 Chinese hamster ovary chromosome aberration test was positive in the presence of low concentrations of rat liver S9-mix and scoring of only 100 cells per sample (Galloway *et al*., 1987a). The same authors also conducted a Sister chromatid exchange assay that was negative (Galloway *et al*., 1987b). Several *in vitro* Comet studies measuring DNA damage were identified using BMP of high purity (98%). Positive results were obtained in urothelial cells (Kong *et al*., 2011; Kong *et al*., 2013), but no genotoxic effect was evident in the hepatocytes (Kong *et al*., 2013). Regarding *in vivo* studies, 2 OECD TG 474 mouse peripheral blood micronucleus tests were reported positive at 400 mg/kg bw/d and 1300 mg/kg bw/d for males and at 600 mg/kg bw/d for females. A positive *in vivo* comet assay (Wada *et al*., 2014) in urinary bladder at of 600 mg/kg bw/d was also identified, with no signs of toxicity. In the same study, negative results were obtained *in vivo* for the liver, similar to observations from the *in vitro* comet study by Kong *et al*. (2013), due to detoxification by glucuronidation in the hepatocytes. No germ cell mutagenicity studies are included. Availability to germ cells: Treinen *et al.* (1989) revealed that BMP leads to reduced fertility, specific effect on female reproductive capacity. BMP is not a selective reproductive toxicant, because these findings are concomitant with general toxicity. However, Bolon *et al*. (1997) showed significantly and dose-response related reduction in follicle numbers in both F0 and F1 mice from the same experiment. Moreover the reduction in follicle numbers occurs also at the mid dose in F1 mice not mediating clear reproductive effects or overt body weight decrease. This indicates that BMP reaches the germ cells.

Based on the above, RAC agreed that BMP should be classified as a germ cell mutagen, Cat. 1B; H340.

In conclusion, 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 from a structurally related and known category-1B mutagen (BMP) is applicable.

Therefore, RAC considers that a **classification for TBNPA as germ cell mutagen Category 2, H341: Suspected of causing genetic defects – is warranted.**

10.2 Carcinogenicity

No data available.

10.2.1 Read-across for mutagenicity and carcinogenicity

10.2.1.1 Hypothesis for the analogue approach

The read-across is based on the report "Category approach for selected brominated flame retardants - preliminary structural grouping of brominated flame retardants" (Wedebye et al., 2016). The subtitle of the report reflects the preliminary structural grouping of all the brominated flame retardants on the Danish market, plus some additional common ones, in total 67. The authors then chose the group of small brominated alkyl alcohols (SBAA) for further investigation, so the main title of the report "Category approach…" refers to the work done on this group while the subtitle "preliminary…" refers to the bigger group initially investigated.

Scientific hypothesis and justification of read-across by characterisation of the analogue approach (according to the ECHA Read-Across Assessment Framework, RAAF scenario 2).

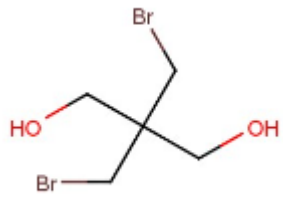
The scientific hypothesis for the read-across is that the chemical structure of TBNPA (CAS-no. 36483-57-5/1522-92-5), BrCC(CBr)(CBr)CO is very similar to BMP that is already classified as Muta. 1B and Carc. 1B (CAS no. 3296-90-0, C(C(CO)(CBr)CBr)O). The structural similarity was recognized by the Danish Environment Protection Agency (DEPA) and published in a report by Wedebye et al. 2016 when **BMP and TBNPA were found to belong to the same (Q)SAR-based clusters for genotoxicity and carcinogenicity**, and described to belong to the group of SBAA. The last member of the group, 2,3-DBPA, is classified with Carc. 1B and Repr. 2. However, this substance does not belong to the same clusters for genotoxicity and carcinogenicity as BMP and TBNPA. For reproductive toxicity the three substances are in separate clusters.

All members of the SBAA group were predicted by a number of (Q)SAR models including the OECD (Q)SAR Toolbox to be positive for carcinogenic and genotoxic properties indicating that they have a carcinogenic potential with a possible mutagenic/genotoxic mode of action (see chapter 3 in Wedebye et al. 2016 for more details on the (Q)SAR predictions).

There are several alerts for mutagenicity and carcinogenicity for SBAAs in the (Q)SAR models applied. For example "aliphatic halogen" is an alert for *in vitro* and *in vivo* mutagenicity and carcinogenicity in the OECD (Q)SAR Toolbox (according to Wedeby et al. (2016), it does not seem that there is one single mechanistic interpretation of this alert in relation to mutagenicity and cancer).

For details on the grouping, see Wedebye et al., 2016.

Table 8: The members of the small brominated alkyl alcohols (SBAA) group:

Chemical name	Synonyms	EC no.	CAS no.	Harmonized classification, CMR	Structural formula
2,2-bis(bromomethyl)propane-1,3-diol (BMP)	Dibromoneopentyl glycol (DBNPG) 2,2-bis-(bromomethyl)-1,3-propanediol Dinol FR-522	221-967-7	3296-90-0	Muta. 1B, H340 Carc. 1B, H350	

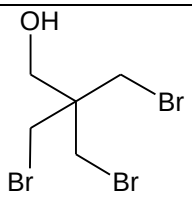
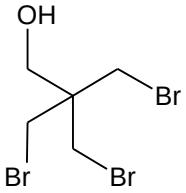
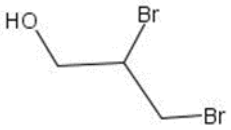
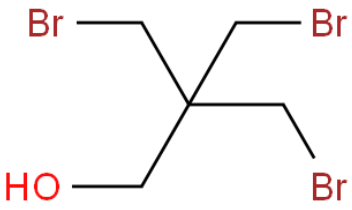
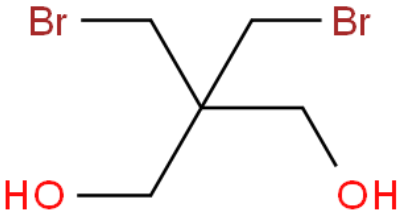
2,2-dimethylpropan-1-ol, tribromo derivative (TBNPA)	2,2-dimethylpropan-1-ol, tribromo derivative, tribromoneopentyl alcohol, 3,3,3-tribromo-2,2-dimethyl-propan-1-ol, FR-513	253-057-0	36483-57-5	-	
3-bromo-2,2-bis(bromomethyl)-1-propanol	3-bromo-2,2-bis(bromomethyl)propan-1-ol Pentaerythritol Tribromide	-	1522-92-5	-	
2,3-dibromo-1-propanol (2,3-DBPA)	2,3-dibromopropan-1-ol 2,3-dibromopropyl alcohol DBP	202-480-9	96-13-9	Carc. 1B, H350 Repr. 2, H361f	

Table 9: Target substance and source substance, Moleculare structures of TBNPA and BMP (Source Danish (Q)SAR database)

	
TBNPA, target substance	BMP, source substance

The Danish report (Wedebye et al., 2016) states that according to (Q)SAR-based clusterings, BMP and TBNPA (the 6,9% impurity in BMP) are in the same (Q)SAR-based clusters for carcinogenicity and genotoxicity. There are genotoxicity study results revealing almost identical properties for BMP and TBNPA.

Table 10: Data matrix for studies relevant for assessing germ cell mutagenicity, Analogue Approach

CAS #	**36483-57-5**	**3296-90-0**
	Target chemical	**Source chemical**
Chemical name	**TBNPA**	**BMP**
Gene mutation in bacteria:		
Bacterial Reverse Mutation Assay (Ames test)		Negative in tested Salmonella strains TA98/100/1535/1537 with and without rat and hamster S9 (Mortelmans et al., 1986; NTP, 1996)
	Positive: Tested in Salmonella strains 1535, TA 1537, TA 98 and	Positive: Tested in TA98/100/1535/1537

	TA 100 with and without rat and hamster S9. Positive in TA 1535 and TA 100 with S9 (study report unnamed, 1996)	Positive in TA1535/100 with hamster S9 Negative with rat S9 Negative without S9 (Unnamed author, 1996b; Zeiger et al., 1992; NTP, 1996)
Gene mutation in mammalian cells:		
Mammalian cell gene mutation test, *in vitro*	Positive: Mutagenicity was seen in mouse lymphoma L5178Y cells with rat S9 (study report unnamed, 2004)	
Mammalian cytogenetic assays, *in vitro*:		
In vitro cytogenicity / Chromosome aberration study in mammalian cells	Positive: Clastogenic chromosomal aberrations in peripheral human lymphocytes with rat S9 (study report unnamed, 2004)	Positive: Chromosomal aberration in Chinese hamster ovary cells with rat S9 (Galloway et al., 1987; NTP, 1996)
Sister Chromatid Exchange Assay		Equivocal: Slight increases in Chinese hamster ovary at toxic levels with S9 (Galloway et al., 1987; NTP, 1996)
Mammalian cytogenetic assays, *in vivo*:		
Micronucleus test (cytogenicity in-vivo).	Negative: TBNPA did not induce micronuclei in mouse femur bone marrow cells (study report unnamed, 2007b)	Positive: BMP was genotoxic in peripheral blood and bone marrow in both male and female mice in *in vivo* micronucleus tests with B6C3F1 mice (NTP, 1996)
DNA damage and repair assays:		
In vitro comet assays		Positive: BMP induced oxidative stress and induced DNA damage in the urothelial cell line of Urotsa cells in two tests (Kong et al., 2011; 2013)
		Negative: No DNA damage was seen in an *in vitro* comet assay with primary hepatocytes (non-target) isolated from male SD rats (Kong et al., 2013)
in vivo comet assay		Positive/Negative: BMP increased DNA damage in urine bladder, but not in liver in SD rats (Wada et al., 2014)
Unscheduled DNA synthesis (UDS) test *in vivo*	Negative: The test material did not induce any marked or toxicologically significant increases in the incidence of cells undergoing DNA synthesisin isolated rat hepatocytes (study report unnamed, 2007a)	
Reproductive toxicity studies:		
National Toxicology Program (NTP) Reproductive Assessment		Positive: BMP impaired fertility in female CD-I mice in both

by Continuous Breeding bioassays (RACB) Archived ovaries from NTP RACB bioassays		generations, no effect on reproductive organ weight or estrual cyclicity (Treinen et al., 1989) Positive: Dose-dependent decreased counts of small and/or growing follicles in CD-I mice (ovaries from the RACB study) (Bolon et al, 1997)
28-day oral repeat dose toxicity study	Negative: No effects on germ cells (Unnamed author, 2015)	

BMP (CAS no. 3296-90-0) showed no evidence of mutagenic activity in Ames´ tests with Salmonella strains TA98, TA100, TA1535 and TA1537, with or without activation from rat liver S9 (Mortelmans et al., 1986; NTP, 1996). There was however clear evidence of positive mutagenic activity from BMP in strains TA1535 and TA100 in the presence of Syrian hamster S9-mix (Unnamed author, 1996b; Zeiger et al., 1992; NTP, 1996). Chromosomal aberration was induced by BMP as breaks in the long arm of the X-chromosome in cultivated Chinese hamster ovary cells (CHO-W-B1) in the presence of metabolic activation (Galloway et al., 1987; NTP, 1996). BMP induced very slight increases in Chinese hamster ovary cells in the *in vitro* Sister Chromatid Exchange Assay at toxic levels with S9, and none withut metabolic activation (Galloway et al., 1987; NTP, 1996). In the two *in vitro* comet assays, BMP induced oxidative stress and induced DNA damage in the urothelial cell line of Urotsa cells (Kong et al., 2011; 2013). No DNA damage was seen in an *in vitro* comet assay with primary hepatocytes (non-target) isolated from male SD rats (Kong et al., 2013). In an *in vivo* comet assay, BMP increased DNA damage in urine bladder, but not in liver in SD rats (Wada et al., 2014). BMP was genotoxic in peripheral blood and bone marrow in both male and female mice in *in vivo* micronucleus tests with B6C3F1 mice (NTP, 1996).

As described in the table above and further in detail in section 10.1 TBNPA was clastogenic in human lymphocytes in vitro and mutagenic in mouse lymphoma cells in vitro. In bacterial reverse mutation assays, mutagenicity was seen. Two in vivo tests with TBNPA were negative.

For BMP there are extensive reproduction toxicity studies indicating that the substance reaches the germ cells (Treinen et al., 1989; Bolon et al, 1997). For TBNPA, there is only a 28 days repeated dose toxicity study available where no effects were observed in germ cells.

For carcinogenicity there is only data for BMP. However it should be noted that TBNPA was a major impurity in the 2-year carcinogenicity study from NTP.

In the 2-year carcinogenicity study on BMP by National Toxicology Program (NTP) (1996) and also published by Dunnick et al. (1997), F-344 rats and B6C3F1 mice were given BMP orally through feed.

Male rats were given 100, 200 or 430 mg BMP/kg bw/day were as female rats were given 115, 230 or 460 mg BMP/kg bw/day, plus a stop exposure at 800 mg BMP/kg bw/day (3 months exposure). Clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas) and at 4 sites in female rats (mammary gland, oral cavity, esophagus and thyroid gland). Dose response relationships between exposure and carcinogenicity were evident for several tumour types, and most cancer-sites are relevant for humans. Survival at the two highest doses in males and females and the male stop-exposure group was significantly lower than controls. Mean body weights of rats receiving the highest dose and the stopexposure group in males were lower than controls (5-15%). Food consumption was generally similar to that by controls, except from stop-exposure males (NTP, 1996).

Male mice were given 35, 70, or 140 mg BMP/kg/day whereas female mice were given 40, 80 or 170 mg

BMP/kg/day.). A clear exposure related carcinogenic effects were also observed at 3 sites in the male (lung, kidney and Harderian gland) and female mice (subcutaneous tissue, lung and Harderian gland). Dose response relationships between exposure and carcinogenicity was evident for several tumour types and most sites of cancer are relevant for humans. Survival of the high dose males and females was significantly lower than that of the controls. Mean body weights of exposed male and female mice were similar to controls throughout the study. Final mean body weights were also generally similar to those of controls. Feed consumption by exposed male and female mice was similar to that by controls (NTP, 1996).

The test material in the study from the NTP, FR-1138®, contains 79% BMP and the following major impurities: 6,9% TBNPA (CAS no 36483-57-5/1522-92-5), 6,6% monobromoneopentyl triol (CAS no 19184-65-7) and other minor impurities (NTP, 1996).

Carcinogenicity was predicted for all the substances in the group small brominated alkyl alcohols when (Q)SAR models were applied (Wedebye et al., 2016).

TBNPA and BMP have almost identical structure, similar physicochemical properties (table 11) and almost similar genotoxicity test results. We assume that the target substance TBNPA and the source substance BMP share the same toxic mode of action for genotoxicity. BMP and other brominated chemicals have been shown to be genotoxic in a spectrum of tests. It is hypothesized that the carcinogenic activity of brominated chemicals is due to genotoxic mechanisms (NTP, 1996). This corresponds to the RAAF scenario 2.

Table 11: Summary of physicochemical properties for TBNPA and BMP

Property	**TBNPA Value** (ECHA dissemination site)	**BMP Value** (ECHA dissemination site)
Physical state at 20°C and 101,3 kPa	Solid, white to off-white flakes	Off white crystalline powder, odourless
Melting point	Melting / freezing point at 101 325 Pa: 68.96°C	Melting / freezing point at 101 325 Pa:109 °C
Boiling point	N.A.	Boiling point at 101 325 Pa:270 °C
Relative density	Relative density at 20C: 2.286	Relative density at 20C: 1.2
Vapour pressure	0 Pa at 25°C	0.002 Pa at 25 °C
Surface tension	N.A.	N.A.
Water solubility	1.93 g/L at 20.1°C	19.4 g/L at 20 °C
Partition coefficient n-octanol/water	Log Kow (Log Pow):2.6 at 22.5°C	Log Kow (Log Pow):1.08

Table 12: Analogue approach – assessment

Assessment element common to all analogue scenarios	**Assessment**	**Score (1-5)**
AE A.1 Source substance	The chemical structure of TBNPA is very similar to BMP	5
AE A.2 Links/differences	Structurally TBNPA and BMP have very similar molecular formulas, differing only in regard to that one of the OH-groups is substituted with Br in TBNPA	4

AE A.3 Source study	The source study is a NTP-study	5
AE A.4 Bias	TBNPA and BMP are in the same (Q)SAR-based clusters for carcinogenicity and genotoxicity	5

Table 13: Analogue approach – scenario 2

Scenario 2 assessment elements (AE): two different compounds with the same type of effect	**Assessment**	**Score (1-5)**
AE 2.1 Compounds	In the NTP 2-year study on BMP the purity was 78,6% Other constituents in the tested flame retardant FR- 1138: 6.9% TBNPA 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane 0.2% pentaerythritol 7.7% dimers and structural isomers	5
AE 2.2 Underlying mechanism, qualitative aspects	The substances share the same genotoxic properties. We assume that the target substance TBNPA and the source substances (BMP) share the same toxic mode of action.	4
AE 2.3 Underlying mechanism, quantitative aspects	The genotoxic responses are similar.	4
AE 2.4 Other compounds	Glucuronidation is the sole route of metabolism of BMP in liver microsomes or primary liver cells of rodents, Rhesus monkey and human. The rate of BMP glucuronidation in rodent cells was 150-fold higher than in human hepatocytes. We assume that this is a detoxification route and that this is the same for TBNPA.	3
AE 2.5 Other effects	The mechanism of carcinogenicity for the source substance is not described beyond genotoxicity. However there is data demonstrating that BMP leads to the induction of oxidative DNA damage, which could be due to the release of bromine.	4

10.2.2 Comparison with the CLP criteria for carcinogenicity

Classification category 1: Known or presumed human carcinogens.

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

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

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

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

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

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

Rationale for proposal for classification in Cat. 1B:

No carcinogenicity study is available for TBNPA. As described above, we propose to read across from the source substance BMP to the target substance TBNPA. RAC states in the recent RAC-opinion for BMP that it considers BMP to be a multi-site carcinogen in two species with tumours of human relevance. Therefore, RAC agreed to classify BMP as Carc. 1B; H350. We propose the same classification for TBNPA.

10.2.3 Conclusion on classification and labelling for carcinogenicity

TBNPA should be classified as Carc. 1B, H350

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There is no carcinogenicity data for the target compound TBNPA. The DS evaluated the carcinogenicity hazard endpoint based on read-across from the source substance BMP. The latter substance has a recent RAC opinion that considers BMP to be a multi-site carcinogen in two species with tumours of human relevance. Therefore, RAC classified BMP as Carc. 1B; H350 (CLH-O-0000001412-86-212/F). The DS proposed the same classification for TBNPA.

Comments received during consultation

Regarding carcinogenicity, three comments were received, one from Industry and two from MSCAs.

Industry

Industry disagreed with the proposed classification for TBNPA as Carc. 1B, based on read-across from the source substance BMP. The reasoning is mainly founded on the following points:

- The two substances have different mutagenic profiles.
- There are significant differences in the structure and physico-chemical properties between the two read across substances.
- The additional hydroxyl group and the fewer bromide groups can make a large difference in biological reactivity.
- The quality of the NTP study, the purity of the BMP used and the underlying mechanism were questioned and, consequently, the score in the RAAF scenario 2, as evaluated by the DS, was incorrectly assigned.
- Previously, it was found, that the kidney pathology in the 28-day toxicity study of TBNPA is different compared to the kidney pathology in the BMP 90-day toxicity study. BMP showed renal papillary degeneration and urinary bladder hyperplasia, whereas TBNPA showed an increase in minimal tubular basophilia, a typical background finding in the rat, considered to be non-adverse. TBNPA had no effects in the bladder, however, the BMP 90-day study observed urinary bladder hyperplasia in 9 out of 10 males. Industry stated that a direct comparison of the results of the 90-day RDT study of BMP with the results of the recently submitted 90-day of TBNPA (Anonymous, 2020) would provide insight about the similarity of the toxicological profile of the two substances.

Furthermore, Industry recognised that, although BMP and TBNPA were found to belong to the same (Q)SAR-based clusters for genotoxicity and carcinogenicity (Wedebye *et al*., 2016), 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. The genotoxicity of BMP (*in vitro* and *in vivo*) added the strength of evidence for classification of BMP as Carc. 1B. Genotoxic carcinogens tend to cross species lines and represent a potential human hazard. This is not the case with TBNPA. In addition, Industry noted that neither for BMP nor for TBNPA human data exist suggesting these SBAA substances are known or presumed human carcinogens. In conclusion, Industry believed that the proposed hazard category for carcinogenicity 1B is too severe and that TBNPA should be classified as Category 2.

MSCA

The first MSCA stated that as the classification for carcinogenicity in the CLH report 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 physico-chemical properties, lack of comparable data regarding reproductive toxicity), which were not discussed in the proposal, raise doubts about the validity of the approach for classification of TBNPA.

The second MSCA noted that, based on the read-across from the source substance BMP classified as Carc. 1B, the same classification for TBNPA is warranted.

Assessment and comparison with the classification criteria

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 presence of limited general toxicity. The opinion was adopted by consensus on June 8, 2018.

Briefly, from the BMP RAC Opinion, "*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); (study conducted by industry; oral)			
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.

Conclusion

Since there are no epidemiological studies available for either the target or the source substance, classification as Carc. 1A is not justified. There are also no animal data on TBNPA. In the animal studies, it is apparent that the source compound, BMP, is a multi-site and multi-species carcinogen. BMP has an adopted RAC opinion as Carc. 1B. Based on the detailed read-across analysis presented above, RAC supports the **classification of TBNPA as Carc 1B, H350: May cause cancer** in agreement with the DS's proposal. Regarding the route of exposure, RAC noted that it should not be specified.

10.3 Reproductive toxicity

10.3.1 Adverse effects on sexual function and fertility

Effects on fertility have not been assessed as no relevant studies are available, except for a 28-day repeated dose toxicity study where no relevant effects were identified.

The study was waived by registrant based on a 28-day oral repeat dose toxicity study in rats with a 14 day recovery period where some relevant reproduction parameters were investigated (Unnamed author, 2015). The results showed no systemic toxicity effects and the No Observed Adverse Effect level (NOAEL) was determined as >500 mg/kg/day (highest dose tested). No treatment related changes in sperm count and motility were observed. Vaginal lavages which were taken early morning during the 3 week period from all females, prior to termination of the animals showed no treatment related changes in the oestrus cycle. In addition, there were no dose related changes in organ weight of ovaries, seminal vesicles, testis, ureter, uterus, vagina in comparison to control animals.

10.3.2 Adverse effects on development

Table 14: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Oral, by gavage, OECD TG 414, SD rats, 20 females/dose	TBNPA 0, 100, 300, 500/(1000) mg/kg bw/d dosed on GD 6-19	In a Prenatal Developmental Toxicity Study in SD rats, the highest dose were reduced from 1000 to 500 mg/kg bw/day due to post dosing toxicity, and two animals were killed due to animal welfare reasons. Transient effects on body weight was seen in the high dose group, and some cases of minor abnormalities in the ossification of pelvic bones were observed in the medium and high dose groups, but in all cases within the historical controls from the test laboratory. Body weight at sacrifice and absolute and relative organ weight data for the parental animals: At 1000 / 500 mg/kg/day mean body weight loss (2%) was observed during Days 6-7 of gestation (after the first dose). On Days 6-9 dams in the high dose group had lower food consumption (4 g/day lower, stat. sign) and body weight gain compared to the controls. The body weight gain was 8, 10 and 6 g in the control, 500 mg/kg/day dose, 1000 mg/kg/day dose, respectively. This was due to both lower gravid uterine weight in the dosed animals and lower body weight gain when the maternal body weight was adjusted for the weight of the uterus. No effects was seen on maternal body weight in the low and medium dose groups. Mean number of live pups (litter size): Embryo-fetal survival was considered to have been unaffected by treatment at 100, 300 or 1000 / 500 mg/kg/day with mean numbers of implantations, resorptions, live young and percentages of sex ratio and pre- and post-implantation loss being similar to control values across all treated groups. Mean litter or pup weight by sex and with sexes combined: Mean placental, male, female and overall fetal weights / litter weight at 100, 300 or 1000 / 500 mg/kg/day were similar to controls and unaffected by treatment. External, soft tissue and skeletal malformations and other relevant: alterations: No dose-related major fetal abnormalities were found. In the medium dose group, there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control (11 fetuses from 7 litters; compared to 4 fetuses from 3 litters in the Controls and 15 fetuses from 12 litters in HCD). In the high dose group there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control in 12 fetuses from 8 litters. This was also within the concurrent Historical Control Data (HCD) range and was considered unrelated to treatment. At 1000 mg/kg/day there was a slightly increased incidence of other minor abnormalities compared to Controls, but all within the historical controls.	Study report unnamed, 2016

10.3.3 Short summary and overall relevance of the provided information on adverse effects on development

In a prenatal developmental toxicity study in SD rats, the highest dose was reduced from 1000 to 500 mg/kg bw/day due to post dosing toxicity. Two animals were killed due to animal welfare reasons. No clear

findings of developmental toxicity was observed. Minor effects on ossification in the medium and high dose groups were within the historical controls. More details in the studies have not been available to the dossier submitter and it was considered not necessary to request the full study report from the registrant.

10.3.4 Comparison with the CLP criteria

The data from the prenatal developmental toxicity study were not considered sufficiently severe to meet the criteria for classification.

10.3.5 Conclusion on classification and labelling for reproductive toxicity

The data for reproductive toxicity is inconclusive. The results from the repeated dose toxicity studies do not warrant classification. The results from the prenatal developmental toxicity study do not warrant classification.

10.4 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

Effects on fertility have not been assessed as no relevant studies are available, except for a 28-day RDT study where no relevant effects were identified.

The required study (OECD TG 421 or 422) was waived by the registrant based on a 28-day oral RDT study in rats with a 14 day recovery period, where some relevant reproduction parameters were investigated (Anonymous, 2015). The results showed no systemic toxicity effects and the No Observed Adverse Effect level (NOAEL) was determined as > 500 mg/kg bw/d (highest dose tested). No treatment related changes in sperm count and motility were observed. Vaginal lavages which were taken early morning during the 3-week period from all females, prior to termination of the animals showed no treatment related changes in the oestrus cycle. In addition, there were no dose related changes in organ weight of ovaries, seminal vesicles, testis, ureter, uterus, vagina in comparison to control animals.

Adverse effects on development

In a prenatal developmental toxicity study in SD rats (see table below), the highest dose was reduced from 1000 to 500 mg/kg bw/d due to post dosing toxicity. Two animals were killed due to animal welfare reasons. No clear findings of developmental toxicity were observed. Minor effects on ossification in the medium and high dose groups were within the historical control data. More details in the studies have not been available to the DS and it was considered not necessary to request the full study report from the registrant.

Overall, the DS considered the data on reproductive toxicity as inconclusive. The results from the RDT studies do not warrant classification. However, the 90-day study is insufficient to fully assess fertility effects, while the results from the prenatal developmental toxicity study do not warrant classification.

Comments received during consultation

Industry agreed with the DS that there are no effects on sexual function or fertility and developmental indices that warrant classification for reproductive toxicity. In addition, Industry stated that the results observed in the 28-day RDT study "are not considered sufficiently severe to meet the criteria for classification".

All 3 MSCAs commenting agreed that the data on TBNPA both for fertility and developmental toxicity are not sufficient for classification. Nevertheless, two MSCAs suggested whether based on structural similarity, read-across from BMP and 2,3-DBPA for reproductive toxicity could be explored.

Assessment and comparison with the classification criteria

Adverse Effects on Sexual Function & Fertility/Development

Table*: pre-natal developmental toxicity study for TBNPA.*

Study/Method/ guideline/deviations if any/ species/groups	Test substance/ dose levels duration of exposure	Results
Study report Anonymous, 2016 Oral, by gavage, OECD TG 414 SD rats (ca 70 days old, 231 to 292 g), 20 females/dose	TBNPA (commercial preparation FR-513) Purity 97.6% Administration to pregnant females via gavage on gestation days 6-19 Doses: 0, 100, 300, 500/(1000) mg/kg bw/d The highest dose was reduced from 1000 to 500 mg/kg bw/d due to post-dosing toxicity after 2-3 doses. Two animals were killed due to animal welfare reasons.	<u>General toxicity</u> Transient effects on body weight were seen in the high dose group. At the high dose (1000 mg/Kg bw/d), *mean body weight loss (2%)* was observed during days 6-7 of gestation (after the first dose) and lower body weight gain later in the study (500 mg/Kg bw/d) compared to controls, even when maternal body weight was adjusted for the weight of the uterus. On days 6-9, dams in the high dose group *had significantly lower food consumption* (4 g/day lower). No effects seen on maternal body weight in the low and medium dose groups. <u>Organs weight:</u> Gravid uterine weight in the dosed animals was lower. <u>Mean number of live pups (litter size)</u>: Embryo-foetal survival was considered to have been at all doses, with mean numbers of implantations, resorptions, live young and percentages of sex ratio and pre- and post-implantation loss being similar to control values across all treated groups. <u>Mean litter or pup weight by sex and with sexes combined</u>: Mean placental, male, female and overall foetal weights/ litter weight, at all doses, were similar to controls and unaffected by treatment. <u>External, soft tissue and skeletal malformations and other relevant alterations</u>: No dose-related major foetal abnormalities were found. <u>Incidences of delayed / incomplete ossification / unossified</u>

		pelvic bones: 300 mg/kg bw/d: 11 foetuses from 7 litters 500 mg/kg bw/d: 12 foetuses from 8 litters Concurrent control: 4 foetuses from 3 litters Historical control data: 15 foetuses from 12 litters

In the 28-day RDT 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, vagina were observed.

In the oral 90-day RDT study in rats (EU B.26./OECD TG 408, Anonymous, 2020), 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 from the 28-days and 90-days RDT studies with TBNPA, RAC considers that classification of TBNPA for **sexual function and fertility is not warranted due to lack of data**.

In addition, based on OECD TG 414 prenatal developmental toxicity study for TBNPA, RAC considers that classification of TBNPA for **developmental effects is not warranted**.

10.5 Specific target organ toxicity-repeated exposure

Table 15: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD TG 407 (Repeated Dose 28-Day Oral Toxicity in Rodents), GLP compliant.	TBNPA (97% pure), Oral by gavage. Corn oil vehicle Doses: 30, 150 or 500 mg/kg/day Dosed daily for 28 days 14 days recovery for the recovery group	Sprague-Dawley rats, male and female A test-article related response was evident in the liver (predominantly at ≥150 mg/kg/day) as indicated by increased organ weight and a correlative microscopic finding of slight minimal centrilobular hypertrophy. The effects were transient and minor changes to liver and kidney weight and salts in blood. Organ weights: A test-substance related response increased liver weight was evident (predominantly at ≥150 mg/kg/day), and a correlative microscopic finding of slight minimal centrilobular hypertrophy were reported. Full or partial recovery were seen at the end of the study. Slightly higher kidney weights were observed in females in the low dose group and in males in the medium dose group. All findings showed full recovery, with the exception of kidney weights which remained slightly high at the end of the recovery period for males in the top dose group. Clinical signs: In the top dose group, frequent incidences of chin rubbing and/or salivation (sometimes reported as excessive) was reported at some point from week 2 in all females and in the majority of males. In one female in the medium dose group, single incidences of chin rubbing and excessive salivation occurred on days 11 and 16, respectively. The signs were occurred following dosing and dissapeared 1-2 hours after. Females receiving 500 mg/kg/day displayed transient unsteady gait approximately 20 minutes after completion of dosing the group on Days 27 and 28 of treatment and one high dose female (No. 54) appeared to be less active than the other females within the group (on Day 28) at the same time. These were transient signs which had resolved by 1 to 2 hours after dosing. Clinical biochemistry findings: At the end of 4 weeks treatment, there was a transient and slightly low sodium concentration (0.98X Control) and slightly high potassium concentration (1.18X Control) in top dose males.	Study report unnamed, 2015 Key study 2 in the dissemination site
14 days oral dosing by gavage. Necropsy on day 15. No guidelines followed	TBNPA (98.4% pure). Oral by gavage. Corn oil vehicle Doses: 0, 100, 300 and 1000 mg/kg/day	Crj: CD(SD) rats, male and female Males receiving 1000 mg/kg/day were killed early on Day 4 of treatment for animal welfare reasons. Urine staining occurred in males and females in the top dose group.	Study report unnamed, 2011 Key study 1 in the dissemination site

30 days feeding study. No GLP/guidelines	TBNPA (98% pure). Given in feed. 0, 10, 30, 100 and 300 mg/kg bw/day nominal in feed	Sprague-Dawley rats, male and female Effects in male rats from 100 mg/kg bw/day included kidney damage and urine bladder hyperplasia. No effects in females.Treatment-related effects were: increase in serum urea nitrogen content in male rats receiving 300 mg/kg/day TBNPA in their diet, and renal tubular damage and generalized hyperplasia of the mucosal lining of urinary bladders of male rats reciving 300 and 100 mg/kg/day of FR-1360 in their diet. No changes were noted in any of the female rats in this study.	Study report unnamed, 1973 Supporting study 3 in the dissemination site

10.5.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Three studies are available, one of them is carried out in accordance with OECD TG 407 with exposure for 28 days followed by a 2 week recovery period (2015). Based on the available studies, the target organs seem to be liver, kidneys and urine bladder after exposure to TBNPA. The effects were mild and reversible, except for one of the studies (2011) where the high dose on day 4 lead to high acute toxicity making it necessary to kill animals for animal welfare reasons. No significant toxic effects were observed.

10.5.2 Comparison with the CLP criteria

The results from the repeated dose toxicity studies do not warrant classification, as there were no significant toxic effects observed. The observations in the repeated dose toxicity studies were not considered sufficiently severe to meet the criteria for classification.

10.5.3 Conclusion on classification and labelling for STOT RE

Based on the available data, no classification is warranted with regards to STOT-RE.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The toxicity of TBNPA following repeated exposure has been evaluated by the DS based on three studies, two oral and one feeding study all in rats.

Study report (Anonymous, 2015)

In a 28-day oral by gavage study (OECD TG 407, GLP) with TBNPA, liver was the target organ of Sprague-Dawley rats as indicated by increased organ weight and slight minimal centrilobular hypertrophy. Moreover, slight changes were observed in the kidney weight as well as in salt blood concentrations. The effects in liver and kidney were fully reversible with the exception of kidney weights, which remained slightly high at the end of the recovery period for males in the top dose group.

Study report (Anonymous, 2011)

In a 14-day oral by gavage study (no guideline study) with TBNPA in Crl:CD(SD) rats urine staining

occurred in males and females in the top dose group (1000 mg/kg bw/d). Males receiving the top dose were killed early on day 4 of treatment for animal welfare reasons.

Study report (Anonymous, 1973)

In a 30-day oral feeding study (no GLP/guideline study) with TBNPA in Sprague-Dawley rats, the effects in male rats from 100 mg/kg bw/d included kidney damage and urine bladder hyperplasia. Additional treatment-related effects were: increase in serum urea nitrogen content in male rats receiving 300 mg/kg bw/d TBNPA in their diet. No changes were noted in any of the female rats in this study.

According to the DS, based on the three available studies, the target organs are the liver, the kidneys and the urine bladder after exposure to TBNPA. The effects were mild and reversible, except for one of the studies (Anonymous, 2011), where the high dose on day 4 lead to high acute toxicity making it necessary to kill animals for animal welfare reasons. No significant toxic effects were observed and the DS proposed no classification for the STOT RE hazard endpoint.

Comments received during consultation

There were two comments received from MSCAs. The first MSCA supported the DS' proposal and stated that although there were effects seen on liver (increased organ weight, minimal centrilobular hypertrophy), kidney (increased organ weight), and urine bladder (hyperplasia of the mucosal lining of urinary bladders), these effects showed full recovery. Thus, no significant toxic effects were observed in the three available studies and therefore no classification is warranted.

The second MSCA noted that based on the available data no significant toxic effects were observed in the animals at low or moderate exposure concentrations of TBNPA and thus no classification is warranted for TBNPA regarding STOT-RE.

Assessment and comparison with the classification criteria

The evaluation of the STOT RE endpoint for the target substance TBNPA is presented below.

***Table**: Repeated dose toxicity studies for TBNPA*

A/A	Species/ Reference	Method/ Test Substance	Results
1	**Study report Anonymous, 2020** Sprague-Dawley rats 10/sex/dose 5/sex/dose recovery groups	*OECD TG 408 Repeated Dose 90-Day Oral Toxicity in Rodents* *Reliability 1 given by the registrant* TBNPA 97% Oral/gavage Doses (mg/kg bw/d): 0, 50, 150, 450 90 days daily dosing 28-d recovery/observation	Body weight: No effects observed Organ weights: No effects observed Food/water consumption: No effects observed Clinical signs: The clinical sign of perineum wet with urine was observed in both males and females at 150 and 450 mg/kg bw/d. By the end of the treatment period, this clinical sign was observed in 9/10 males and 7/10 female rats of the main group and 4/5 male and 3/5 female rats of the recovery group. The finding was fully reversible at the end of the recovery period. The rest of the observations of all tested groups were comparable to the vehicle control group. No behavioural changes observed. Haematological/Coagulation effects: There were no TBNPA exposure related changes in

		period NOAEL = 50 mg/kg bw/d for m NOAEL = 150 mg/kg bw/d for f	haematology, prothrombin time and activated partial thromboplastin time in males and females. Urinalysis: No treatment related findings in urine parameters Clinical biochemistry: An increase in blood urea nitrogen at 450 mg/kg bw/d and in creatinine at ≥ 150 mg/kg bw/d in males was considered as test item related. This increase was associated with microscopic findings of increased eosinophilic droplets in the tubular epithelium consisting tubular casts in kidneys. These findings reversed at the end of recovery period. Gross pathology: No effects seen Histopathology: Test item related microscopic changes were noted in kidneys and urinary bladder in males. 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. No neoplastic findings were observed. Mortality: None observed
2	**Study report Anonymous, 2015** Sprague-Dawley rats 5/sex/dose CLH report	*OECD TG 407 Repeated Dose 28-Day Oral Toxicity in Rodents, GLP compliant.* *Reliability 1 given by the registrant* TBNPA 97% Oral/gavage Doses (mg/kg bw/d): 0, 30, 150, 500 28 days daily dosing 14-d recovery/observation	Body weight: no effects observed Organ weights: Increased liver weight (predominantly at ≥ 150 mg/kg bw/d), and a correlative microscopic finding of slight minimal centrilobular hypertrophy were reported. Full or partial recovery were seen at the end of the study. Slightly higher kidney weights were observed in females in the low dose group and in males in the medium dose group. All findings showed full recovery, with the exception of kidney weights which remained slightly high at the end of the recovery period for males in the top dose group. Food/water consumption: no effects observed Clinical signs: (500 mg/kg bw/d) Chin rubbing and/or salivation in all females and most males was reported. Females also displayed unsteady gait. All signs occurred following dosing and disappeared 1-2 hours after.

		period NOAEL = 500 mg/kg bw/d both for m and f	Haematological effects: No clear treatment related findings Urinalysis: After exposure to TBNPA (≥ 30 mg/kg/ bw/d) a dose-related increase in urinary volume and slightly high total protein and glucose output in males was seen. All of these findings showed full recovery at the end of the 2-week recovery Clinical biochemistry: (500 mg/kg bw/d) Transient slightly low sodium concentration (0.98X control) and slightly high potassium concentration (1.18X control). Gross pathology: No effects Histopathology: No effects Mortality: None observed
3	**Study report Anonymous, 2011** Crj: CD(SD) rats 5/sex/dose CLH report	*No Guideline* *Repeated Dose 14-Day Oral Toxicity in Rodents), No GLP* *Reliability 2 given by the registrant* TBNPA 98.4% Oral/gavage Doses (mg/kg bw/d): 0, 100, 300 and 1000 14 days daily dosing Day 15 sacrifice No post exposure observation period NOAEL (m) = 300 mg/kg bw/d both for m and f Due to mortality at the top dose NOAEL (f) = 1000 mg/kg bw/d	Body weight: no major changes except for changes in males receiving the top dose, where some of them lost weight. Organ weights: Liver, kidneys and spleen were examined with no effects observed. Food/water consumption: no effects observed Clinical signs: Post dose salivation and chin rubbing was observed on occasion in the majority of animals at all dose levels. Haematological effects: Not examined Clinical biochemistry: Not examined Gross pathology: Enlargement of the liver with associated dark areas was seen in one female at 1000 mg/kg bw/d. Histopathology: Not examined Mortality: Males receiving 1000 mg/kg bw/d (top dose) were killed on day 4 of treatment for animal welfare reasons. Last in-life signs included, abnormal gait, unresponsive, underactive, flat posture, prostrate posture and high levels of urine staining in all males at this dosage. Macroscopic examination revealed abnormal contents and pallor of the jejunum in three of the five animals, but there were no other consistent macroscopic observations recorded for these animals.
4	**Study report Anonymous, 1973** Sprague-Dawley rats	*No Guideline* *Repeated Dose 30-Day Oral Toxicity in Rodents), No GLP*	Body weight: No effects observed Organ weights: Liver, kidneys and spleen were examined with no effects observed.

	5/sex/dose CLH report	*Reliability 2 given by the registrant* TBNPA 98% Oral/feeding Doses (mg/kg bw/d): 0, 10, 30, 100, 300 30 days daily dosing No post exposure observation period NOAEL (m) = 30 mg/kg/ bw/d for based on the kidney and urinary bladder effects NOAEL (f) = 300 mg/kg/ bw/d	Food/water consumption: No effects observed Clinical signs: Not examined Haematological effects: No effects observed Clinical biochemistry: Decrease in SGPT (300 and 100 mg/kg bw/d) and increase in blood urea nitrogen in males (300 mg/kg bw/d) Gross pathology: Effects in male rats from 100 mg/kg bw/d included kidney damage and urine bladder hyperplasia. No effects in females. Hyperplasia of transitional epithelium of the urinary bladder (100 and 300 mg/kg bw/d; males); eosinophilic clumping of the cytoplasm and darkening of nuclei in cortical tubular epithelial cells. Regenerative changes in the large epithelial tubular cells. Histopathology: Changes in kidneys and urinary bladders (male at 300 and 100 mg/kg bw/d). Mortality: No mortality occurred

The recently submitted sub-chronic 90-day toxicity study (Anonymous, 2020) () by the oral route (EU B.26./OECD TG 408) in rats, as requested by ECHA had the goal 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 SD 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 a 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/d) or urine composition/output (increased urinary volume and total protein and glucose output in males at 500 mg/kg bw/d) occurred and a slight increase in kidney weight was evident in both sexes (predominantly at ≥ 150 mg/kg bw/d). 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. However, doses of 1000 mg/kg bw/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 bw/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 bw/d, histologic changes in kidneys and urinary bladder were noted in male rats. No changes were noted in any of the female rats.

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 RDT data for TBNPA is adequate for evaluation and RAC bases the evaluation of the STOT RE endpoint on the TBNPA RDT 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.

10.6 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in thos dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not relevant

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15 ANNEXES

ANNEX I to the CLH report

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

EC Number: **253-057-0**

CAS Number: **36483-57-5 and 1522-92-5**

Index Number: **603-RST-VW-Y**

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Version number: 1.2 **Date: 14 June 2019**

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No studies available.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated.

3.2 Acute toxicity - dermal route

Not evaluated.

3.3 Acute toxicity - inhalation route

Not evaluated.

3.4 Skin corrosion/irritation

Not evaluated.

3.5 Serious eye damage/eye irritation

Not evaluated.

3.6 Respiratory sensitisation

Not evaluated.

3.7 Skin sensitisation

Not evaluated.

3.8 Germ cell mutagenicity

The studies below are included in the REACH registration. When searching in Toxnet, two additional Ames tests from 1990 seem to have been conducted. These tests are neither published nor available from the registrant, and hence not included here.

3.8.1 In vitro data

3.8.1.1 Unnamed (2004)

Study reference:

Study report unnamed, 2004: https://echa.europa.eu/registration-dossier/-/registered-dossier/6484/7/7/2

Key study 1 for in vitro genetic toxicity in the registration.

Detailed study summary and results:

In vitro cytogenicity/chromosome aberration study in mammalian cells (lymphocytes: Peripheral human lymphocytes).

GLP and OECD Test Guideline (TG) 473 was followed (In Vitro Mammalian Chromosomal Aberration Test). The purpose of the in vitro chromosomal aberration test is to identify substances that cause structural chromosomal aberrations in cultured mammalian cells.

Reliability index made by the registrant: 1

- *number of replicates:* Main test 1: 1A+1B+1C+1D, Main test 2: 2A
- *number of doses, justification of dose selection:* Following a range-finder test with doses of 33-3250 µg/ml with and without metabolic activation with S9-mix, the doses in the main studies ranged from 100 to 2000 µg/ml with and without metabolic activation. There were 5 – 7 doses in each study, e.g. 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml in Main study 1.
- *positive and negative control groups and treatment:* Positive control was MMC (Mitomycin C, a clastogen active without metabolic activation) and cyclophosphamide (CP, a clastogen requiring metabolic activation). Negative control (solvent only) was DMSO. 3hr exposure, 24 hr fixation.
- *details on slide preparation: number of metaphases analysed:* Not given in the registration
- *justification for choice of vehicle:* According to test guideline (TG)
- *solubility and stability of the test substance in vehicle if known:* -
- *description of follow up repeat study:* -
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):* The study is GLP compliant.

Test substance

- The test material FR-513 (CAS no. 36483-57-5) used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity 97%
- Impurities: Dibromoneopentyl glycol < 0.1%, which does not affect the classification
- Batch number: Not specified

Administration/exposure

- *lymphocytes:* Peripheral human lymphocytes, with and without metabolic activation
- *Type and composition of metabolic activation system:*
  - Aroclor-1254 induced rat liver S9-mix
  - Quantity not given in the registration
- *Test concentrations, and reasoning for selection of doses if applicable:* Following a range-finder test with doses of 33-3250 µg/ml with and without metabolic activation with S9-mix, the doses in the main studies ranged from 100 to 2000 µg/ml with and without metabolic activation. There were 5 – 7 doses in each study, e.g. 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml in Main study 1:
- Range finder test: control: (DMSO), 33, 100, 333, 1000, 3250µg/ml (-S9-mix) (3hr, 24 hr, 48 hr exposure; 24hr, 24hr,48 hr fixation) ; control (DMSO), 33, 100, 333, 1000, 3250µg/ml (+S9-mix) (3hr exposure; 24hr fixation)
  Main test 1: control (DMSO), 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 100, 333, 666, 1000, 1250, 1500, 2000 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1A: control (DMSO), 100, 333, 666, 1000, 1050, 1100, 1150, 1200 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 100, 333, 666, 1000, 1050, 1100, 1150, 1200 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1B: control (DMSO), 333, 1000, 1010, 1020, 1030, 1040, 1050 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1C: control (DMSO), 333, 666, 1000 µg/ml, MMC-C (0.5 µg/ml) (-S9-mix)(3hr exposure, 24 hr fixation); control (DMSO), 333, 666, 1000, 1020, 1050, 1100, 1200 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 1D: control (DMSO), 100, 300, 600, 800, 1000, 1020, 1030, 1050, 1100 µg/ml, CP (15 µg/ml) (+S9-mix)(3hr exposure, 24 hr fixation)
  Main test 2: control (DMSO), 17, 66, 100, 126, 150, 200 µg/ml, MMC-C (0.2 µg/ml) (-S9-mix)(24 hr exposure, 24 hr fixation); control (DMSO), 1, 3, 10,17, 66, 100 µg/ml, MMC-C (0.1 µg/ml) (-S9-mix)(48 hr exposure, 48 hr fixation); control (DMSO), 100, 167, 666, 1000, 1250, 1500, 2000 µg/ml, CP (15 µg/ml) (+S9-mix)(3 hr exposure, 3 hr fixation)
  Main test 2: control (DMSO), 17, 66, 100, 126, 150, 200 µg/ml, MMC-C (0.2 µg/ml) (-S9-mix)(24 hr exposure, 24 hr fixation); control (DMSO), 1, 3, 10,17, 66, 100 µg/ml, MMC-C (0.1 µg/ml) (-S9-mix)(48 hr exposure, 48 hr fixation)
  Main test 2A: see in materials and methods* (no more info given in the registration)
- *Vehicle:* DMSO.
- *Statistical methods:* Not given in the registration.

Results and discussion

- FR-513 was found to be clastogenic in the presence of metabolic activation, and at the highest test substance concentration (1000 microgram/ml) in the absence of metabolic activation. FR-513 has the potential to disturb mitotic processes and cell cycle progression.
- *Cytotoxic concentrations with and without metabolic activation:* yes (cytotoxic at 1000µg/ml, cell lysis at 3250µg/ml). Cytotoxicity seen as low as at 100 µg/ml, with metabolic activation.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* See above.
- *Concurrent negative (solvent/vehicle) and positive control data*
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,:* Data was not presented in such detail in the registration. However the conclusion was that the test substance was positive clastogenic in the presence and in the absence of metabolic activation.

3.8.1.2 Unnamed (2004)

Study reference:

Study report unnamed, 2004: *In vitro* gene mutation study in mammalian cells:
https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/2/?documentUUID=1de76583-e4ca-4077-803c-7bf6dc92ad76
Key study 2 for in vitro genetic toxicity in the registration.
Reliability index made by the registrant: 1

Detailed study summary and results:

Test type

Mammalian cell gene mutation assay (gene mutation) following OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Test). GLP compliant.

Mouse lymphoma L5178Y cells.

- *number of replicates:* 2 experiments, both with and without metabolic activation
- *number of doses, justification of dose selection:* Dose range finding test (without/with metabolic activation): Solvent control, 33, 100, 333, 1000, 3250 µg/ml. In the main studies, concentrations varied from 10 to 535 µg/ml, and up to 8 dose groups pluss two solvent controls, e.g. 10, 50, 100, 200, 300, 400, 500 µg/ml in experiment 1 (without metabolic activation).
- *positive and negative control groups and treatment:* Positive control methyl methane sulfonate/cyclophosphamide
- *details on slide preparation* -
- *number of metaphases analysed* -
- *justification for choice of vehicle*: According to test guideline
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations):* GLP compliant

Test substance

- The test material FR-513 (CAS no. 36483-57-5) used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity 97%
- Impurities: Dibromoneopentyl glycol < 0.1%, which does not affect the classification
- Batch number: Not specified

Administration/exposure

- Mouse lymphoma L5178Y cells
- *Type and composition of metabolic activation system:*
  - with and without rat liver microsomal enzymes (S9-fraction).
  - *quantity* – not given
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations:*

  Dose range finding test (without/with metabolic activation): Solvent control, 33, 100, 333, 1000, 3250 µg/ml

Experiment 1 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 200, 300, 400, 500, positive control (MMS)
Experiment 1 (with metabolic activation 8%): first solvent control, second solvent control, 50, 100, 200, 300, 375, 450, 500, positive control (CP)
Experiment 2 (without metabolic activation): first solvent control, second solvent control, 10, 50, 100, 225, 250, 300, 325, 350, positive control (MMS)
Experiment 2 (with metabolic activation 12%): first solvent control, second solvent control, 100, 200, 300, 350, 400, 470, 500, 535, positive control (CP)

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation*: The test substance was cytotoxic with and without metabolic activation from concentration of 333 µg/plate.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation: Mutant frequencies:* In the absence of S-9 mix FR-513 did not induce a significant increase in mutant frequencies in the first experiment. This result was confirmed in a repeat experiment with modifications in the duration of the time treatment from 3 to 24 hours. FR-513 was mutagenic in the test system with incubations in the presence of metabolic activation. The presence of S9-mix in both tests resulted an increase in mutation frequencies more than threefold and outside the labs historical data. The increases were considered biologically relevant and FR-513 is considered mutagenic in vitro.

3.8.1.3 Unnamed (1996)

Study reference:

Study report unnamed, 1996: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/2/?documentUUID=e115ea71-ed77-449f-9943-6bca66b27e3a

Key study 3 for in vitro genetic toxicity in the registration.

Reliability score made by the registrant: 1

Detailed study summary and results:

Bacterial reverse mutation assay: In vitro gene mutation study in bacteria (Ames test)

The study was done according to OECD TG 471, and GLP, on S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (with and without metabolic activation from rodent S-9 mix - treated with Aroclor 1254). Strains TA98 and TA1537 are capable of detecting frameshift mutagens, strains TA100 and TA1535 are capable of detecting base-pair substitution mutagens.

The study was done according to OECD TG 471.

Test substance

- The test material FR-513 used in the study is equivalent to the substance identified in the CLH dossier.
- *Degree of purity:* 98%
- *Impurities (or a note that the impurities do not affect the classification):* Not given in the registration

Administration/exposure

- S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
- *Type and composition of metabolic activation system:*
  - hamster S-9 mix (treated with Aroclor 1254)
- *Test concentrations, and reasoning for selection of doses if applicable:* The concentration was 0, 5, 50, 500, 5000 µg/plate in the preliminary toxicity determination (with and without metabolic activation), and 0, 15, 50, 150, 500, 1500 µg/plate in the main test (with and without metabolic activation).

Results and discussion

- 5000 µg/plate in the preliminary toxicity determination was toxic so the highest concentration was set to 1500 µg/plate in the main test.
- Toxicity was observed in the preliminary test at the concentration of 5000 µg/plate.
- Large, dose-related increases in revertant colony numbers were observed in both mutation tests with strains TA 1535 and TA 100, at concentrations between 15 and 500 µg/plate, but this was only observed with metabolic activation.
- DMSO used as negative control. Postive control was N-Ethyl-N-nitro-N-nitrosoguanidine, 9-Amonoacridine, 2 Nitrofluorene, 2-Aminoantracene, Congo red (CAS no. 573-58-0) which demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

3.8.2 Animal data

3.8.2.1 Study report (2007)

Study reference:

Study report unnamed, 2007: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/3 Key study 1 for *in vivo* genetic toxicity in the registration.

Detailed study summary and results:

Unscheduled DNA Synthesis (UDS) test with rat liver cells (liver hepatocytes) in vivo. GLP and OECD TG 486 was followed.

Test substance

- FR 513 equivalent to the substance identified in the CLH dossier
- *Degree of purity*: Not given in the registration.
- *Impurities (or a note that the impurities do not affect the classification):* Not given.

Test animals

- Sprague-Dawley rats (CD (Ctr;CD (SD) IGS BR) strain)
- *No. of animals per sex per dose:* range finder: 2 (1 male; 1 female) (2 males 0 females); main test 1: 4 per dose (males); main test 2: 4 per dose (males)
- *Age and weight at the study initiation:* 6-9 weeks old. Weight: male 200 gr-243 gr (start of experiment)

Administration/exposure

- *Doses/concentration levels, vehicle, rational for dose selection.* All animals were dosed once. In the range finding test the dose was 2000 mg/kg. In the main studies the dose was 670 and 2000 mg/kg bw.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Archis oil
- *Details on test system and conditions, and details on route of administration, exposure:* Oral by gavage
- *Duration of study, frequency of treatment, sampling times and number of samples:* Treatment: 16 hr (experiment 1); 2 hr (experiment 2)
- *Positive and negative (vehicle/solvent) control data:* Positive control 2- Acetamididofluorene (2AAF) at 50 mg/kg bw, and Sym-Dimethylhydrazine dihydrochloride (NDHC) at 40 mg/kg bw.
- *Methods of slide preparation:* The coded slides were scored using an automated image analysis system linked to a computer programe (Grain) which followed the UKEMS guidelines for statistical analysis.

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* Negative: The test material did not induce any marked or toxcologically significant increases in the incidence of cells undergoing DNA synthesis in isolated rat hepatocytes following in vivo exposure for 2 or 16 hr. Therefore the test material was considered to be non-genotoxic under the conditions of the study.
- *Concurrent positive control data:* Both positive controls produced marked increases in the incidence of cells in repair and the vehicle control groups gave acceptable values for net nuclear grain counts.
- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable.* Administration of the test substance in the range finding study produced toxicity in the dosed animals manifested as ataxia, lethargy, red colored urine (no deaths). Lethargy and ataxia was also seen in the main studies.

3.8.2.2 Unnamed (2007)

Study reference:

Study report unnamed, 2007: |https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/7/3/?documentUUID=ca22dcb7-3231-4dd3-95f8-44fad85f4c68

Key study 2 for in vivo genetic toxicity in the registration.

Detailed study summary and results:

In vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus

Reliability score made by the registrant: 1

Test type

OECD test guideline 474 is relevant, but the study was done prior to the guideline. No major deviations from the guideline

Test substance

- FR 513, equivalent to the substance identified in the CLH dossier
- *Degree of purity:* 98.1%
- *Batch number:* 39084

Test animals

- *Species/strain/sex:* NMRI mice/male and female
- *No. of animals per sex per dose:* In total 81 animals (45 males and 36 females). Ten animals (5 males, 5 females) per dose.
- *Age and weight at the study initiation:* Initial age at the start of acclimatisation: 8-9 weeks (males), 11-12 weeks (females). Initial body weight at start of treatment: Males mean value 32.9g (SD ± 1.9 g); Females mean value 31.3 g (SD ± 2.7 g)

Administration/exposure

- *Doses/concentration levels, vehicle, rational for dose selection:* Preliminary test: 2000, 1500, 1000, 500,400, 300 (mg/kg b.w) main test: 300, 150, 75 (mg/kg bw. On the day of the experiment, the test item was formulated in DMSO+corn oil (30%-70%). The vehicle was chosen to its relative non-toxicity for the animals. All animals received a single standard volume of 10 mL/kg body weight orally.
- *Vehicle*: Identification, concentration and volume used, justification of choice of vehicle (if other than water): DMSO+corn oil (30%-70%)
- *Positive and negative (vehicle/solvent) control data:* Positive control substance(s): CPA; Cyclophosphamide (>98%); Dosing: 40 mg/kg b.w ; volume administration: 10 mL/kg b.w

Results and discussion

- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal):* FR-513 did not induce micronuclei as determined by this micronucleus test with femur bone marrow cells of the mouse. The % micronuclei was 0.085, 0.110 and 0.125 at dose 75, 150, 300 mg/kg bw 24 hours post-treatment.

3.9 Carcinogenicity

No studies available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Unnamed (2015)

Study reference: Study unnamed, 2015: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Reliability score made by the registrant: 1

Effect on developmental toxicity: Via oral route

Detailed study summary and results:

Test type

28-day oral repeat dose toxicity study, OECD Guideline 407 compliant

Test substance

- 97% TBNPA
- The impurities do not affect the classification
- Batch number 2119-72-01

Test animals

- SD rats
- 5 males and 5 females
- *Age and weight at the study initiation:* Approximately 7-8 weeks, males 201-246 g (males), 154-188 g (females)

Administration/exposure

- *Route of administration:* oral (gavage)
- *duration and frequency of test/exposure period:* 28 days
- *doses/concentration levels, rationale for dose level selection:* 30, 150, 500 mg/kg bw/day
- *control group and treatment:* yes, concurrent vehicle
- *historical control data if available:* available
- *vehicle:* corn oil

Results and discussion

The results showed no systemic toxicity effects and the No Observed Adverse Effect level (NOAEL) was determined as >500 mg/kg/day (highest dose tested). No treatment related changes in sperm count and motility were observed. Vaginal lavages which were taken early morning during the 3 week period from all females, prior to termination of the animals showed no treatment related changes in the oestrus cycle. In addition, there were no dose related changes in organ weight of ovaries, seminal vesicles, testis, ureter, uterus, vagina in comparison to control animals.

3.10.1.2 Unnamed (2016)

Study reference: Study unnamed, 2016: https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/9/3

Reliability score made by the registrant: 1

Effect on developmental toxicity: Via oral route

Detailed study summary and results:

Test type: Prenatal Developmental Toxicity Study

OECD Guideline 414 (Prenatal Developmental Toxicity Study). GLP compliant.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* FR-513, equivalent to the substance identified in the CLH dossier
- *Degree of purity:* 97.6%

Test animals

- *Species/strain/sex:* Sprague-Dawley M/F, (Crl:CD(SD) strain)
- *No. of animals per sex per dose:* 20 females per dose
- *Age and weight at the study initiation:* Approximately 70 days old, 231 to 292 g.

Administration/exposure

- *Route of administration*: oral (gavage)
- *duration and frequency of test/exposure period:* Pregnant females received daily doses via gavage from day 6 to 19 during the pregnancy.
- *doses/concentration levels, rationale for dose level selection:* Dose levels were 100 (low) and 300 (medium) mg/kg bw/day. In addition there was a dose group of 1000 mg/kg bw/day, but this had to be reduced to 500 mg/kg/d (high) shortly after commencement of the treatment due to signs of post dosing toxicity, after 2-3 doses. Two females in the top dose group were killed for animal welfare reasons. Some animals received 500 mg/kg bw/d (high) already from the start. Se dosing details below in table.
- *control group and treatment:* The control group received corn oil at the same volume and duration as the treated groups.
- *vehicle:* Corn oil
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* The homogeneity and stability of formulations during storage were determined and ensured.

Description of test design: Study was done according to OECD TG 414 and GLP.

The study consisted of one control and three treated groups identified as follows:

Group	Treatment	Dose # (mg/kg/day)	Number of animals Female	Animal numbers Female
1	Control	0	20	1-20
2	FR-513	100	20	21-40
3	FR-513	300	20	41-60
4	FR-513	1000 / 500 @	20	61-80

\# Expressed in terms of material as supplied

@ 1000 mg/kg/day dosed 23-25 May. From 26 May 2016 the dose level of 500 mg/kg/day was used. Female numbers 61 -63 received 1000 mg/kg/day during Days 6-8 of gestation, female numbers 64 -72 received 1000 mg/kg/day during Days 6-7 of gestation, and female numbers 73-77 received 1000 mg/kg/day on Day 6 of gestation only. Female numbers 78-80 received the lowered dose of 500 mg/kg/day only from Day 6 to Day 17 of gestation.

Results and discussion:

- *time of death during the study and whether animals survived to termination:* Animals were killed on Day 20 after mating for reproductive assessment and detailed fetal examination. In two females toxicity signs were so severe as to require termination after 2 or 3 doses, on Day 7 or Day 8 of gestation
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals:* At 1000 / 500 mg/kg/day mean body weight loss was observed during Days 6-7 of gestation (after the first dose). Later in the study, dams in the high dose group had lower food consumption and body weight gain compared to the controls. This was due to both lower gravid uterine weight in the dosed animals and lower body weight gain when the maternal body weight was adjusted for the weight of the utreus. No effects was seen on maternal body weight in the low and medium dose groups.
- *Food consumption:* Was slighty affected only in the top dose group on days 6-9, and afterwards similar to controls.
- *clinical observations: description, severity, time of onset and duration:* Signs of chin rubbing and salivation were observed in animals of all treated groups, however, this was considered to relate to general distaste of the formulation rather than any effect of toxicity. Signs of toxicity was observed after treatment of pregnant female Sprague Dawley rats with FR-513 at 1000 mg/kg/day from Day 6 of gestation with marked post dosing signs including underactive/unresponsive behaviour, partially closed eyelid(s), unsteady muscle reaction and prostrate posture following one to three doses. In two females these findings were so severe as to require termination after 2 or 3 doses, on Day 7 or Day 8 of gestation. Following the reduction of the high dose level to 500 mg/kg/day (after the third day of dosing) the signs of toxicity were less marked or no longer

apparent. No signs of toxicity was observed in the low and medium dose groups during dosing, and no macroscopic findings were made in the dams during necropsy in any of the dose groups.

- *haematological and clinical biochemistry findings if available:* Not available
- *necropsy findings:* No macroscopical findings in any dose group.

Pups/litters (per dose):

- *mean number of live pups (litter size)*
  Embryo-fetal survival was considered to have been unaffected by treatment at 100, 300 or 1000 / 500 mg/kg/day with mean numbers of implantations, resorptions, live young and percentages of sex ratio and pre and post-implantation loss being similar to control values across all treated groups.
- *mean litter or pup weight by sex and with sexes combined:* Mean placental, male, female and overall fetal weights / litter weight at 100, 300 or 1000 / 500 mg/kg/day were similar to controls and unaffected by treatment.
- *external, soft tissue and skeletal malformations and other relevant alterations:* No dose-related major fetal abnormalities were found. In the medium dose group, there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control (11 fetuses from 7 litters; compared to 4 fetuses from 3 litters in the Controls and 15 fetuses from 12 litters in HCD). In the high dose group there was a slightly increased incidence of the minor abnormalities delayed / incomplete ossification / unossified pelvic bones compared to concurrent control in 12 fetuses from 8 litters. This was also within the concurrent Historical Control Data (HCD) range and was considered unrelated to treatment. At 1000 mg/kg/day there was a slightly increased incidence of other minor abnormalities compared to Controls, but all within the historical controls.

3.10.2 Human data

3.10.2.1

No data available.

3.11 Specific target organ toxicity – single exposure

Not evaluated.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Unnamed, 2015

Study reference:

Study report unnamed, 2015 https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Detailed study summary and results:

Test type

OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents), GLP compliant.

Key study 2 in the registration. Reliability 1 given by the registrant

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-513 (TBNPA)
- Degree of purity: 97%
- Batch number 2119-72-01

Test animals

- Sprague-Dawley rats/male and female
- Number of animals 33 males and 33 females. 5 males and 5 females per dose.
- Age of the main study and recovery animals at start of treatment: Approximately 7 to 8 weeks of age.
- Weight range of the main study and recovery animals at the start of treatment Males: 201 to 246 g Females: 154 to 188 g

Administration/exposure

- Oral gavage
- Dosing once daily for 28 days
- Doses: 30, 150 or 500 mg/kg/day of FR-513. Rationale for dose level selection not found
- Post exposure observation period: Two weeks
- Vehicle: Corn oil. Volume dose 5 mL/kg body weight. control group and treatment: Vehicle at the same volume dose as the treated groups

- Homogeneity and stability of FR-513 in corn oil formulations (at nominal concentrations of 6 and 100 mg/mL) was confirmed
- Actual doses: 30, 150 or 500 mg/kg/day of FR-513 (equivalent to 6, 30 and 100 mg/mL using a dose volume of 5 mL/kg body weight)
- Statistical methods not given

Results and discussion

- body weight and body weight changes: no effects observed.
- organ weights: A test-substance related response increased liver weight was evident (predominantly at ≥150 mg/kg/day), and a correlative microscopic finding of slight minimal centrilobular hypertrophy were reported. Full or partial recovery were seen at the end of the study. Slightly higher kidney weights were observed in females in the low dose group and in males in the medium dose group. All findings showed full recovery, with the exception of kidney weights which remained slightly high at the end of the recovery period for males in the top dose group.
- food/water consumption: no effects observed
- clinical signs: In the top dose group, frequent incidences of chin rubbing and/or salivation (sometimes reported as excessive) was reported at some point from week 2 in all females and in the majority of males. In one female in the medium dose group, single incidences of chin rubbing and excessive salivation occurred on days 11 and 16, respectively. The signs were occurred following dosing and dissapeared 1-2 hours after. Females receiving 500 mg/kg/day displayed transient unsteady gait approximately 20 minutes after completion of dosing the group on Days 27 and 28 of treatment and one high dose female (No. 54) appeared to be less active than the other females within the group (on Day 28) at the same time. These were transient signs which had resolved by 1 to 2 hours after dosing.
- no effect on motor activity
- ophthalmologic findings: not available
- haematological findings: Blood was collected on day 29 for the main study animals and on day 15 for the recovery animals: No clear treatment related findings.
- clinical biochemistry findings: At the end of 4 weeks treatment, there was a transient and slightly low sodium concentration (0.98X Control) and slightly high potassium concentration (1.18X Control) in top dose males
- gross pathology findings: no effects
- histopathology findings: no effects

- no mortality observed

3.12.1.2 Unnamed, 2011

Study report unnamed, 2011 https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/6484/7/6/2/?documentUUID=bdfdd675-2048-4263-be0c-16a9ce3e7a9b

Key study 1 in the registration. Reliability 2 given by the registrant.

Detailed study summary and results:

Test type

No guideline applicable.

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-513 (TBNPA)
- Degree of purity: 98.4%

Test animals

- Crj: CD(SD) rats, male and female (a total of 25 male and 25 female)
- 5 male and 5 female per dose
- The rats were ordered at 29 to 35 days of age and within a weight range of 118 to 145 g for
- males and 108 to 135 g for females. The animals were allowed to acclimatise to the conditions described below for 12 days before treatment commenced. For those animals selected for this study, their age at the start of treatment was 41 to 47 days and their bodyweights were in the range of 209 to 272 g for males and 161 to 197 g for females
- All animals were subject to a necropsy.

Administration/exposure

- route of administration: daily oral gavage
- The test substance was administered daily over a period of 14 consecutive days. The necropsy procedures were completed on Day 15.

  Males receiving 1000 mg/kg/day were prematurely sacrificed on day 4 of treatment for animal welfare reasons.
- Doses were 0, 100, 300 and 1000 mg/kg/day.
- post exposure observation period: no

- vehicle: corn oil, 5 mL/kg bodyweight

Results and discussion

- body weight and body weight changes: no major changes except for changes in males receiving the top dose, where some of them lost weight.
- Liver, kidneys and spleen were examined and weighed. Organ weight findings including organ / body weight ratios: no effects observed
- food/water consumption: no effects
- haematological findings: not examined
- clinical biochemistry findings: not examined
- three out of five females receiving 1000 mg/kg/day to show urine staining during the treatment period (Day 4, 11 and 15), with this sign also observed at macroscopic examination
- gross pathology findings: Enlargement of the liver with associated dark areas was seen in one female receiving 1000 mg/kg/day
- histopathology findings: not examined
- post dose salivation and chin rubbing was observed on occasion in the majority of animals at all dose levels
- Males receiving 1000 mg/kg/day (top dose) were killed early on day 4 of treatment for animal welfare reasons. Last in-life signs included, abnormal gait, unresponsive, underactive, flat posture, prostrate posture and high levels of urine staining in all males at this dosage. Macroscopic examination revealed abnormal contents and pallor of the jejunum in three of the five animals, but there were no other consistent macroscopic observations recorded for these animals.

3.12.1.3 Unnamed, 1973

Study report unnamed, 1973

Supporting study 3 in the registration. Reliability 2 given by the registrant

Detailed study summary and results:

Test type

Non-GLP study

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier. Unnamed constituent FR-1360 (TBNPA)
- Purity 98%
- Dibromoneopentyl glycol 1.4% ; Tetrabromoneopenthane 0.6%

Test animals

- Male and female Sprague-Dawley rats
- 5 animals per sex per dose
- Age at the study initiation: 6-7 weeks

Administration/exposure

- route of administration – oral in feed
- 30 days dosing in feed
- 10, 30, 100 and 300 mg/kg bw/day nominal in diet
- vehicle: not given
- control group and treatment: there was a control group
- test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation: no details given
- actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable: not given
- statistical analysis of variance and Dunnett´s test

Results and discussion

- body weight and body weight changes: No effects observed
- No effects observed on food consumption. Water consumption not examined.
- No clinical signs were recorded.
- sensory activity, grip strength and motor activity assessments (when available): Not examined
- ophthalmologic findings: Not examined
- On day 24 hematologic evaluations and urinalysis were conducted on the male and female rats from the control groups and groups receiving 300 mg/kg/bwclinical biochemistry findings: decease in Serum Glutamic Pyruvic transaminase (SGPT) (300 and 100 mg/kg/day) and increase in Blood Urea Nitrogen in males (300 mg/kg/day)At the termination of the test, blood samples were collected from all the rats for determination of serum urea nitrogen, alkaline phosphatase and glutamic pyruvic transaminase. No hematological effects observed
- Necropsy was conducted. At necropsy, a complete gross pathological examination was conducted and the weights of heart, liver, kidney, testes and brain were recorded.

- Treatment-related effects were: increase in serum urea nitrogen content in male rats receiving 300 mg/kg/day FR-1360 in their diet, and renal tubular damage and generalized hyperplasia of the mucosal lining of urinary bladders of male rats reciving 300 and 100 mg/kg/day of FR-1360 in their diet. No changes were noted in any of the female rats in this study.
- No mortality occurred

3.12.2 Human data

No data available.

3.13 Aspiration hazard

Not evaluated.

4 ENVIRONMENTAL HAZARDS

Not evaluated.