Committee for Risk Assessment

RAC

Annex 1

Background document

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

2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate; trimethylolpropane triacrylate

EC Number: 239-701-3

CAS Number: 15625-89-5

CLH-O-0000006856-61-01/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 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

17 September 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-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate; trimethylolpropane triacrylate

EC Number: 239-701-3

CAS Number: 15625-89-5

Index Number: 607-111-00-9

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Version number: v1 **Date: February 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYL-2-[[(1-OXOALLYL)OXY]METHYL]-1,3-PROPANEDIYL DIACRYLATE; 2,2-BIS(ACRYLOYLOXYMETHYL)BUTYL ACRYLATE; TRIMETHYLOLPROPANE TRIACRYLATE

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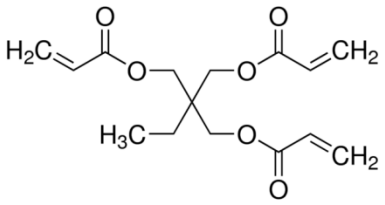
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate
Other names (usual name, trade name, abbreviation)	Trimethylolpropane triacrylate (TMPTA)
EC number (if available and appropriate)	239-701-3
CAS number (if available)	15625-89-5
Molecular formula	$C_{15}H_{20}O_6$
Structural formula	
Molecular weight or molecular weight range	296.3157
Degree of purity (%) (if relevant for the entry in Annex VI)	> 80%

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)
2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate EC n°239-701-3 CAS n°15625-89-5	> 80%	Skin Irrit. 2 H315 Eye Irrit. 2 H319 Skin Sens. 1 H317	

No impurities or additives may contribute to the classification of TMPTA.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	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	607-111-00-9	2,2-bis(acryloyloxymethyl)butyl acrylate trimethylolpropane triacrylate	239-701-3	15625-89-5	Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1	H315 H319 H317	GHS07 Wng	H315 H319 H317			D
Dossier submitters proposal	607-111-00-9	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate; trimethylolpropane triacrylate	239-701-3	15625-89-5	**Add** Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	**Add** H351 H400 H410	**Add** GHS08 GHS09	**Add** H351 H410		**Add** M=1 M=1	
Resulting Annex VI entry if agreed by RAC and COM	607-111-00-9	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate; trimethylolpropane triacrylate	239-701-3	15625-89-5	Carc. 2 Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H315 H319 H317 H400 H410	GHS07 GHS08 GHS09 Wng	H351 H315 H319 H317 - H410		M=1 M=1	D

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	Data conclusive but not sufficient for classification	No
Flammable gases (including chemically unstable gases)	Hazard class not applicable (liquid)	-
Oxidising gases	Hazard class not applicable (liquid)	-
Gases under pressure	Hazard class not applicable (liquid)	-
Flammable liquids	Data conclusive but not sufficient for classification	No
Flammable solids	Hazard class not applicable (liquid)	-
Self-reactive substances	Hazard class not assessed in this dossier	-
Pyrophoric liquids	Data conclusive but not sufficient for classification	No
Pyrophoric solids	Hazard class not applicable (liquid)	-
Self-heating substances	Hazard class not assessed in this dossier	-
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	No
Oxidising liquids	Data conclusive but not sufficient for classification	No
Oxidising solids	Hazard class not applicable (liquid)	-
Organic peroxides	Hazard class not assessed in this dossier	-
Corrosive to metals	Hazard class not assessed in this dossier	-
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 Already classified as Skin Irrit. 2 – H315	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier Already classified as Eye Irrit. 2 – H319	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier Already classified as Skin Sens. 1 – H317	No
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Harmonised classification proposed: Carc. 2 – H351	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed: Aquatic Acute 1 – H400, M-factor: 1 Aquatic Chronic 1 – H410, M-factor: 1	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Trimethylolpropane triacrylate (TMPTA) is currently classified according to CLP regulation (CLP00).

The substance was on the Corap list for substance evaluation by France in 2014. After the initial year of Substance evaluation, the need to update the current harmonized classification of TMPTA for the carcinogenicity endpoint was identified. Moreover, additional data were requested to Registrants to clarify concerns that include mutagenicity (Comet assay on bone marrow and liver; OECD 489) and aquatic toxicity (fish acute toxicity test; OECD 203). The registration dossiers were updated in October 2017 to provide the requested information. The Substance Evaluation was concluded in October 2018 (conclusion document to be published) and it was identified based on the fish study provided during the SEv process that classification for Aquatic toxicity is justified. Mutagenicity data, including the study generated during the SEv process have also been included in the present report as it is relevant information to consider for a comprehensive evaluation of carcinogenicity.

RAC general comment

Trimethylolpropane triacrylate (TMPTA) is an industrial chemical used as an intermediate in the production of weather-resistant coatings and dry ink cartridges for professional use. There are no consumer uses. It is a clear liquid with a low vapour pressure (0.1 Pa at 20 °C) and a water solubility of 0.5 g/L. Based on the physico-chemical properties, the main excretion route is expected to be via kidney and was confirmed in a toxicokinetic study in rats and mice with dermal and i.v. application. In addition, exhalation was shown to be a significant route of excretion in this toxicokinetic study with radiolabelled TMPTA. This is due on the suspected degradation of TMPTA to acrylic acid by blood esterase and the known degradation of acrylic acid to CO_2. Its structure is shown below.

TMPTA has an Annex VI entry with the harmonised classification Skin Irrit. 2; H315, Eye Irrit. 2; H319 and Skin Sens. 1; H317. The need to update the current classification for carcinogenicity was identified in the CoRAP process by France (dossier submitter). The Substance Evaluation was concluded in October 2018. Mutagenicity data, including one study generated during the evaluation process, have been included in the CLH report as relevant information for a comprehensive evaluation of carcinogenicity.

Based on a fish study provided during the evaluation process in was concluded that classification for Aquatic toxicity is also justified.

No other endpoints were open for consultation.

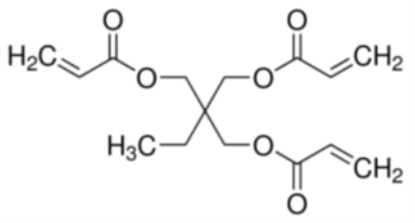


Figure: Chemical structure of TMPTA.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

For carcinogenicity endpoint, there is no requirement for justification that action is needed at Community level.

Concerning classification for aquatic toxicity, justification that action is needed at Community level is required.

Unconsistent self-classifications are reported in the ECHA inventory database whereas available data show that TMPTA has aquatic toxicity property that is not currently harmonised and justify a harmonised classification and labelling.

C&L inventory (checked on 25th June 2019) reported that

- 152/2690 notifiers classify TMPTA as Aquatic Acute 1 – H400;
- 158/2690 notifiers classify TMPTA as Aquatic chronic 1 – H410, 18/2690 notifiers classify TMPTA as Aquatic chronic 2 – H411, and 51/2690 notifiers classify TMPTA as Aquatic chronic 3 – H412

5 IDENTIFIED USES

TMPTA is used in industrial applications of coating and inks in dry process and in polymerisation in the polymer industry. TMPTA is also used by professional for indoor printing with ink cartridges in dry process. There is no consumer uses (use advised against) (ECHA website, 2018).

6 DATA SOURCES

Information described in this CLH report are based on the REACH registration dossier and bibliographic search ended in September 2018.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Clear liquid	(Registration dossier, IUCLID 6)	Visual inspection, purity not given
Melting/freezing point	< -20 °C at 1013.25 hPa	Kintrup (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Boiling point	> 390 °C at 1013.25 hPa	Kintrup (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Relative density	1.1086 at 20 °C	Frischmann (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Vapour pressure	0.1 Pa at 20 °C	Kintrup (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Surface tension	51 mN/m at 20 °C	Dreyer (2014) (Registration dossier, IUCLID 6)	Measured value, purity not given
Water solubility	0.5 g/L at 20°C	Frischmann (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Partition coefficient n-octanol/water	Log Kow (Pow): 4.35 at 25°C	Dreyer (2014) (Registration dossier, IUCLID 6)	Calculated, purity not given
Flash point	194.5 °C at 1013.25 hPa	Kintrup (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Flammability	Non flammable	Kintrup (2010) (Registration dossier, IUCLID 6)	Statement The substance can be mixed in water without development of gas.
Explosive properties	Non explosive	(Registration dossier, IUCLID 6)	Statement There are no chemical groups associated with explosive properties present in the substance.
Self-ignition temperature	385 °C at 1013.25 hPa	Kintrup (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Oxidising properties	Non oxidizing	(Registration dossier, IUCLID 6)	Statement There are no chemical groups associated with oxidising properties present in the substance.

Property	Value	Reference	Comment (e.g. measured or estimated)
Granulometry	Stable in organic solvents	(Registration dossier, IUCLID 6)	Statement
Stability in organic solvents and identity of relevant degradation products	The substance does not dissociate in water	Frischmann (2010) (Registration dossier, IUCLID 6)	The measured conductivity is similar to the conductivity of water, purity not given
Dissociation constant	122 mPa.s (dynamic) at 20 °C	Frischmann (2010) (Registration dossier, IUCLID 6)	Measured value, purity not given
Viscosity	Clear liquid	(Registration dossier, IUCLID 6)	Visual inspection, purity not given

8 EVALUATION OF PHYSICAL HAZARDS

Trimethylolpropane triacrylate (TMPTA) has no physical properties warranting classification under CLP.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 6: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
rats and mice (male) F344/N (rats) and B6C3F (mice) Intravenous injection (single administration): - Nominal doses (rats) [14C]: 9.4 mg/kg bw Dermal application (single administration): - Nominal doses (rats) [14C]: 1.7, 15.2 and 130 mg/kg bw - Nominal doses (mice) [14C]: 1.2 mg/kg bw dermal strip experiment: - Nominal doses (rats) [14C]: 124 mg/kg bw Pre-exposure dermal study (2 applications): - Nominal doses (rats) [first unlabeled]: 151 mg/kg bw at 24 hour interval Stability was confirmed during a dermal carcinogenicity experiment; using GC, recovery rates were (with minor exception) in the range above 85% (mostly	**Absorption**: Absorbed dose (rats, dermal, 1.7 mg/kg) Total Absorbed Dose: 55.1% Urine: 28%, cage wash: 0.9%, faeces: 2.5%, expired air (CO_2): 13.1% Skin: acetone extractable: - ; non extractable: 8.5% Selected tissue: 0.4%, carcass: 1.7% Total unabsorbed Dose (appliance and skin wash): 34.8% Total Dose Recovery: 90% Absorbed dose (rats, dermal, 15.2 mg/kg) Total Absorbed Dose: 32.7% Urine: 12.1%, cage wash: 0.9%, faeces: 1.2%, expired air (CO_2): 4.9% Skin:acetone extractable: 8.0%; non extractable: 3.3% Selected tissue: 1.0%, carcass: 1.2% Total unabsorbed Dose (appliance and skin wash): 57.0% Total Dose Recovery: 89.7% Absorbed dose (rats, dermal, 130 mg/kg) Total Absorbed Dose: 18.7% Urine: 3.0%, cage wash: 0.1%, faeces: 0.2%, expired air (CO_2): 1.4% Skin: acetone extractable: 10.4%, non extractable: 3.2%	2 (reliable with restrictions) key study experimental result **Test material (EC name): TMPTA**	NTP (2005)

Method	Results	Remarks	Reference
near 100). NTP protocol. Purpose and Guidelines for Toxicokinetics Studies within the National Toxicology Program. Environmonmental Health Perspectives, Vol. 105, No. 5, pp. 468-471, 1997.	Selected tissue: 0.2%, carcass: 0.3% Total unabsorbed Dose (appliance and skin wash): 76.1% Total Dose Recovery: 94.8% <u>Absorbed dose (rats, dermal, 2 applications of 151 mg/kg)</u> Total Absorbed Dose: 25.4% Urine: 5.8%, cage wash: 0.4%, faeces: 0.4%, expired air (CO_2): 3.4% Skin: acetone extractable: 8.5%, non extractable: 2.6% Selected tissue: 0.7%, carcass: 3.6% Total unabsorbed Dose (appliance and skin wash): 65.3% Total Dose Recovery: 90.7% ➔ Absorption increases with increased number of exposure <u>Absorbed dose (mice, dermal, 1.2 mg/kg)</u> Total absorbed: 75% Urine: 16.5%, cage wash: 2%, faeces: 5.6%, expired air: 18.2% Skin: 30.8%, tissues: 0.2%, carcass: 1.7% Total non absorbed (appliance + skin wash): 20.9% Total dose recovery: 95.9% ➔ Dermal absorption higher in mice than in rats **Distribution**: < 6% in tissues and carcass. High kidney:blood ratios in dermal dosed rats Intravenous application showed tissue:blood ratio below 0.7 for all tissues. High skin:blood ratios in mice. **Excretion**: - i.v. injection (rats, 9.4 mg/kg): urine (48.6%), feces (8.7%) and exhaled CO_2 (20.1%) - dermal application (rats): urine (28.0% (1.7 mg/kg) - 3.0% (130 mg/kg)), exhaled CO_2 (13.1% (1.7 mg/kg) - 1.4% (130 mg/kg)), faeces (0.2-2.5%) - dermal application (mice): urine and expired CO2 (both about 16-18%), faeces (5.6%) **Details on metabolites** (tape stripping experiment): Following 72 hours of exposure, acetone extracts of the stripped, sliced skin were prepared and analyzed by HPLC; the major peak, accounting for approximately 73% of the radiolabel, was associated with trimethylolpropane triacrylate. Two more peaks accounted for 14% and 10% of the radiolabel. Preliminary stability studies showed that TMPTA was chemically unstable in whole blood: no parent TMPTA was reliably		

Method	Results	Remarks	Reference
	measured in blood. No bioaccumulation potential.		
In vitro percutaneous absorption through human skin Human female breast skin, 4 donors, 8 replicates Neat substance: 910 g/L (9098 µg/cm^2) Exposure: 8 hours Sampling duration: 24 hours GLP, OECD guideline 428	Absorption (receptor fluid + receptor compartment wash + skin membrane + stratum corneum excluding first 2 tape strips) = 0.60 +/- 0.26%	1 (reliable without restrictions) key study experimental result **Test material (EC name): TMPTA**	Anonymous, 2015

GI absorption:

No experimental data are available with TMPTA in regards to oral absorption. Following the REACH guidance document 7c, the physicochemical properties of TMPTA (molecular weight of ~296 g/mol and water solubility of 500 mg/L) are favourable to oral absorption. According to Danish QSAR database, an absorption from gastro intestinal tract for a dose of 1 mg is estimated at 95% and for a dose of 1000 mg at 50%. Additionally, acute oral toxicity studies (Anonymous 1972, Anonymous 1980) showed deaths indicating some evidence of bioavailability. Finally, considering the irritating properties of TMPTA, oral absorption may be enhanced by irritation of the gastro-intestinal tract.

Absorption by inhalation:

No experimental data are available with TMPTA in regards to inhalative absorption. According to the REACH guidance document 7c, physicochemical data enable qualitative judgments of the toxicokinetic behavior. The limited vapour pressure and water solubility property of the substance are not in favour of respiratory absorption. Furthermore, the result of acute inhalation toxicity studies shows no toxicity up to the vapour saturation concentration (Anonymous, 1976) indicating either a low absorption of TMPTA and/or a low toxicity potential after inhalation.

Dermal absorption:

An *in vivo* study was performed in rats and mice (NTP, 2005). This study shows an inverse dose dependent dermal absorption rate. It is shown that a total of 18.7% of the 130 mg/kg dose, 32.7% of 15.2 mg/kg dose and 55.1% of 1.7 mg/kg dose were absorbed in rats after a single dermal application. Due to the irritative potential of the substance, it may also be possible that absorption increased after repeated exposure to the substance. When rats were pre-exposed to 151 mg/kg bw of no radiolabeled TMPTA 24h prior to the same dose of radiolabeled TMPTA, the dermal absorption was 25.4%. This confirms that repeated dose exposure of TMPTA can enhance dermal absorption (25.4% absorbed after 2 applications of 151 mg/kg vs 18.7% after single application of 130 mg/kg). Total recoveries ranged from 89.7 to 94.8% which is lower than the minimal recovery (95%) recommended by EFSA guidance (2017). Therefore, the actual absorption may be underestimated based on this study in rats. In mice dermally exposed to 1.2 mg/kg, 75% of TMPTA was absorbed. The total recovery was 95.9%. In conclusion, significant amounts of TMPTA are absorbed if applied dermally in rats and mice. Dermal absorption seems to be higher in mice compared to rats.

A recent *in vitro* percutaneous absorption study through human skin (Anonymous, 2015) was also available with TMPTA. Breast skins were exposed to TMPTA (890 g/L) for 8 hours. Samples used to estimate dermal absorption were collected until 24 hours after application. The authors concluded to an absorption of the neat

substance at 0.60% when considering the amount in the receptor fluid, the receptor compartment wash, the skin membrane and the stratum corneum excluding the first 2 tape strips. According to EFSA guidance (EFSA, 2017), one standard deviation should be added to the mean dermal absorption value leading to a dermal absorption of 0.8%. Only one high non-diluted concentration was used in this study. Without any detailed information on the typical formulations on the market, it is not possible to assess the relevance of the obtained dermal value from this study to the expected use condition of TMPTA.

Distribution and accumulative potential:

The physico-chemical information (molecular weight, lipophilicity and water solubility) indicates that TMPTA could in principle be distributed to many tissues.

The distribution of TMPTA was investigated in the NTP dermal study (NTP, 2005). Less than 6% of radioactivity was recovered in selected tissues and residual carcass in rats and mice. In rats dermally exposed to a single dose from 1.7 to 130 mg/kg of TMPTA, tissue: blood ratios were below 1 with exception of the kidney: blood ratio (approximately 3.3-11.1). Bladder: blood ratio was also elevated in the group pretreated with 151 mg/kg. Intravenous application to 9.4 mg/kg showed tissue: blood ratios below 0.7 for all tissues, and even 72h after IV application, most of the not-excreted dose could be found in the blood. According to the NTP (2005), the elevated kidney: blood ratio, seen only after dermal exposure, may be due to urine in the making at the time of necropsy. Similar to rats, very little radiolabel was associated with most of the tissues 72h after dosing in mice dermally exposed to TMPTA. However, the bladder, kidney, liver and skin had a tissue: blood ratio > 1. In a tape stripping experiment in rats exposed to 124 mg/kg, only about 1.5% of the radiolabeled was removed by tape stripping after 30-minute or 72-hour exposure; therefore high concentrations in the stratum corneum can be excluded. Very low levels (<1% of the applied dose) were also found in the *in vitro* percutaneous study on human skin. No accumulation potential is expected after TMPTA exposure.

Metabolism:

The major compound found in the tape stripping experiment is the parent component (approximately 73%) followed by two unknown signals in the in HPLC chromatogram. These both metabolites count for a fraction of 10% and 14%. The type of metabolites was not specified in the NTP (2005) study. Preliminary stability studies to the NTP (2005) study indicated that [14C]-trimethylolpropane triacrylate was chemically unstable in whole blood of rats after a single intravenous injection (no parent TMPTA was reliably measured in blood 0.08 hours to 72 hours after injection). Due to its chemical structure, the degradation of TMPTA by blood esterase to acrylic acid, along with trimethylolpropane diacrylate and monoacrylate and/or trimethylolpropane is possible and expected to be the peaks observed in the HPLC chromatogram. In addition, according to Danish QSAR database, TMPTA is not expected to be a CYP2C9 or CYP2D6 substrate.

Reactivity:

Reactivity to nucleophilic molecules (e. g. thiol or amine groups of proteins) can be expected considering the alpha, beta-unsaturated nature of TMPTA.

Excretion:

Based on the physico-chemical information (molecular weight and water solubility), main excretion via kidney can be expected. In addition, based on the suspected degradation of TMPTA to acrylic acid and the known degradation of acrylic acid to CO_2, exhalation is also expected to be a significant route of excretion. These major routes of excretion are confirmed within the NTP (2005) study. After IV administration in rats, [C14]-TMPTA was mainly measured in urine (48%), then in expired CO_2 (20.1%) and faeces (8.7%). After dermal application in rats, the major route of elimination was also the urine (3-28%), followed by expired CO_2 (1.4-13%) and faeces (0.2-2.5%). Excretion was dose-dependent, with higher elimination rate after

lower doses tested. In mice, TMPTA was eliminated at similar amount in urine and expired CO_2 (16-18%) and then in faeces (5.6%).

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

There is no kinetics data by oral or inhalation routes. Species-difference in term of dermal absorption is suggested based on the *in vivo* study in rats and mice and the *in vitro* study in human skin, with the highest absorption value in mice (75%) and the lowest in humans (0.8%). However, some cautions should be taken with the results obtained from the *in vitro* study on human skins since only one high concentration was tested.

It can be assumed that the substance is distributed to many different tissues, but unlikely to accumulate. Only little TMPTA can be found in the tissue and carcass 72h after application. First step in metabolism shows parent component and two metabolites (suspected to be acrylic acid and the alcohol trimethylol propane). Reactivity to nucleophilic molecules (e. g. thiol or amine groups of proteins) can be expected considering the alpha, beta-unsaturated nature of TMPTA.

Elimination of the substance, mainly via urine, exhaled air and faeces, was reported. Excretion via exhaled air is in favour of a conversion into acrylic acid and later to CO_2.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not assessed in this dossier.

10.2 Skin corrosion/irritation

Not assessed in this dossier. TMPTA is currently classified as Skin Irrit. 2 – H315.

10.3 Serious eye damage/eye irritation

Not assessed in this dossier. TMPTA is currently classified as Eye Irrit. 2 – H319.

10.4 Respiratory sensitisation

Not assessed in this dossier.

10.5 Skin sensitisation

Not assessed in this dossier. TMPTA is currently classified as Skin Sens. 1 – H317.

10.6 Germ cell mutagenicity

Table 7: 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** **Reliability**
Tests in bacteria				
bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Positive control substance(s) included equivalent or similar to OECD Guideline 471	TMPTA Purity not stated	Test concentrations: 0.2, 2, 20, 500 µg/plate for the incorporation assay and 5 mg for the spot test.	Negative for TA 1535, TA 1537, TA 98 and TA 100; with and without metabolic activation. Cytotoxicity: growth inhibition in the spot assay, not reported in the incorporation assay Vehicle, negative and positive controls valid	Anonymous (1976) 2 (reliable with restrictions)
Bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Positive control substance(s) included Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	TMPTA Purity > 70%	Test concentrations: 20, 100, 500, 2500 and 5000 µg/plate for all strains (1st experiment) and 0-4000 µg/plate for TA1535 (2nd experiment)	Negative for TA 1537, TA98 and TA100 with metabolic activation. Cytotoxicity: slight decrease for TA 98 above 2500 µg/plate Positive (from 500 µg/plate – without clear dose-dependent relationship) for TA 1535 with metabolic activation; not cytotoxic Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation; not cytotoxic Negative control, vehicle control and positive controls valid.	Anonymous (1989a) 2 (reliable with restrictions)
Bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Positive control substance(s) included	TMPTA Purity = 79%	Test concentrations: 100;333;1,000;3,333;6,667; 10,000 µg/plate	Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation and with metabolic activation (rat S9); not cytotoxic Vehicle and positive controls valid Positive for TA 1535 with hamster S9	Cameron *et al.* (1991) 2 (reliable with restrictions)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference** **Reliability**
Equivalent or similar to OECD Guideline 471				
bacterial reverse mutation assay (pre-incubation method) *Salmonella typhimurium* (TA98 and TA100) or *Escherichia coli* (WP2 *uvrA*/pKM101) (met. act.: with and without) Positive control substance(s) included equivalent or similar to OECD Guideline 471	TMPTA Purity > 78%	Test concentrations: 1,500;3,000;5,000;7,500 to 10,000 µg/plate	Negative for all tested strains. Slight toxicity at 10,000 µg/plate with S9 No information on cytotoxicity Vehicle and positive controls valid	NTP (2012) 2 (reliable with restrictions)
Test on mammalian cells				
Gene mutation assay mouse lymphoma L5178Y cells (met. act.: with and without) Positive control substance(s) included equivalent or similar to OECD Guideline 476	TMPTA Purity not stated	Preliminary toxicity test: 0.004875 to 5 nL/mL (without S9); 0.004875 to 40 nL/mL (with S9). Mutation test: Test 1: 0.078 to 1.25 nL/mL without S9; 0.150 to 2.50 nL/mL with S9 Test 2: 0.150 to 1.00 nL/mL without S9; 1.250 to 10.00 nL/mL with S9 Test 3: 1.00 to 2.5 nL/mL without S9; 2.00 to 20 nL/mL with S9	Positive for mouse lymphoma L5178Y cells without metabolic activation in all 3 trials With metabolic activation: - Unconclusive in the first trial - Negative in the second trial - Positive in the third trial at 20 nl/mL (with 4.8% relative growth) Cytotoxicity: yes Vehicle, negative and positive controls valid	Anonymous (1979) 2 (reliable with restriction)
Gene mutation assay Chinese hamster Ovary (CHO) (met. act.: without) Positive control substance included Equivalent or similar to OECD Guideline 476 Chromosomal	TMPTA Purity not stated	Test concentrations: 0, 0.2, 0.6, 0.7 µg/mL	Negative for gene mutation in CHO without metabolic activation Positive for chromosome aberrations in CHO cells without metabolic activation Cytotoxicity: yes Vehicle and positive controls valid	Moore *et al.* (1989) 2 (reliable with restrictions)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference** **Reliability**
aberrations were also examined in CHO in this publication.				
Gene mutation assay mouse lymphoma L5178Y cells (met. act.: without) Positive control substance(s) included equivalent or similar to OECD Guideline 476 Induction of micronucleus and chromosome aberrations were also investigated in L5178Y cells without metabolic activation.	TMPTA Purity not stated	Test concentrations: 0, 0.6, 0.65, 0.7 µg/mL	Positive (exclusive induction of small colonies) for mouse lymphoma L5178Y cells without metabolic activation Positive for induction of micronucleus and chromosomal aberrations in L5178Y without metabolic activation Cytotoxicity: yes Vehicle positive controls valid	Moore *et al.* (1989) Dearfield (1989) 2 (reliable with restrictions)
Gene mutation assay mouse lymphoma L5178Y cells (met. act.: with and without) Positive control substance(s) included equivalent or similar to OECD Guideline 476	TMPTA Purity = 79%	Test concentrations (M): without S9 : $3x10^{-7}$; $1.1x10^{-6}$; $1.8x10^{-6}$; $2.5x10^{-6}$; $3.3x10^{-6}$ With S9 : $3.34x10^{-5}$; $5.19x10^{-5}$; $7.05x10^{-5}$; $9.28x10^{-5}$; $1.1x10^{-4}$	Negative for mouse lymphoma L5178Y cells with metabolic activation Positive for mouse lymphoma L5178Y cells without metabolic activation Cytotoxicity: yes Vehicle and positive controls valid	Cameron et al. (1991) 2 (reliable with restriction)
Mammalian chromosome aberration test Lymphocytes: primary cell cultures from human peripheral blood (met. act.: with and without) Positive control substance(s) included OECD Guideline	TMPTA Purity = 84.6%	Preliminary experiment: 23.1, 45.7, 92.6, 185, 370, 740, 1480 and 2960 µg/mL for the preliminary experiment both with and without S9 mix Main experiment: 1) 0.78, 1.56, 3.13, 6.25, 12.5, 18.75, 25 and 37.5 µg/mL for the first experiment without S9 mix 2) 1.56, 3.13, 6.25, 12.5, 18.75, 25, 37.5 and 50 µg/mL for the first experiment with S9 mix	Positive without metabolic activation at 18.75 µg/ml (first experiment) and ≥ 9.38 µg/mL (second experiment) Positive with metabolic activation from 37.5 µg/ml (first experiment) and from 28.13 µg/mL (second experiment) Cytotoxicity: observed at all concentrations in both first and second experiments at conc. ≥ 12.5 µg/mL without S9 mix and at conc. ≥ 25 µg/ml (first	Anonymous (2005) 1 (reliable without restriction)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference Reliability
473 EU Method B.10 EPA OPPTS 870.5375		3) 3.13, 6.25, 9.38, 12.5, 18.75 and 28.13 µg/mL, for the second experiment without S9 mix 4) 9.38, 18.75, 28.13, 37.5, 50 and 75 µg/mL, for the second experiment with S9 mix	experiment) or ≥ 37.5 µg/ml (second experiment) with S9 mix Vehicle and positive controls valid	

Table 8: 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 Reliability
micronucleus assay (chromosome aberration) Positive control substance(s): No The study was performed as part of subchronic dermal studies described in the repeated dose section. Male and female mice were dermally exposed to the test substance 5 times per week for 14 or 28 weeks. Blood was collected from the retroorbital sinus and stained for analysis of micronuclei and NCE/PCE ratio.	TMPTA Purity = 80%	Mouse (B6C3F) for the 14 week study Genetically modified (FVB tg.AC hemizygous) mice for the 6 month study male/female dermal 0, 0.75, 1.5, 3, 6, 12 mg/kg (nominal conc.)	Negative (male/female) Toxicity: decrease in the percentage of NCEs among total erythrocytes in the 6-month study only Vehicle controls valid: yes Positive controls valid: not included	NTP (2005) 3 (not reliable)
micronucleus assay (chromosome aberration) Positive control substance: Cyclophosphamide; 50 mg/kg bw; oral route OECD Guideline 474 EU Method B.12 EPA OPPTS 870.5395 GLP compliant	TMPTA Purity = 87.9%	Mouse (Swiss Ico: OF1 (IOPS Caw)) male/female oral: gavage; single administration 437.5, 875 and 1750 mg/kg bw (for males) or 500, 1000 and 2000 mg/kg bw (for females) (nominal conc.)	Negative (male/female) Toxicity: only in males (piloerection at 875 mg/kg bw; 2 deaths and piloerection in surviving animal at 1750 mg/kg bw) Vehicle and positive controls valid No proof of bone marrow exposure	Anonymous (2006) 2 (reliable with restrictions)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference** **Reliability**
Mouse alkaline Comet assay GLP, similar to OECD 489	TMPTA Purity = 80.2%	CD-1 females mice (6/dose; 3 for positive control group) Intravenous; in PEG 400 5, 10, 20 mg/kg; 2 doses 24h intervals; sampling 30 minutes after last dose. Organs: liver and bone marrow	Negative in liver Statistically significant increased of mean tail intensity values in bone marrow at 5 and 10 mg/kg according to the authors. Toxicity: some clinical signs including rapid and/or gasping respiration, staggering, lethargy, dark eyes in some animals at the two highest doses. No effect on body weight. No clinical chemistry, macroscopic and microscopic findings.	Anonymous (2018); Reliability not evaluable considering the uncertainties linked to PEG 400

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

GENETIC TOXICITY *IN VITRO*

Gene mutation in bacteria:

Four studies are available to assess the induction of gene mutations by TMPTA in bacteria. Negative results were found with *S. typhimurium* (TA 100, TA 1537 and TA 98) or *E. Coli* (WP2 *uvrA*/pKM101) with and without metabolic activation. Positive response was reported for TA 1535 only in the presence of metabolic activation in two out of the 4 studies (with rat S9 and hamster S9, respectively). In the first test (Anonymous, 1989a), the detected increase varied between a factor of 1.6 and 4.8 with no dose dependency - may be a reflection of toxicity at the higher concentrations. In the second test (Cameron, 1991), the detected increase was about 2.5 fold and was also not dose-dependent. The biological relevance of this finding is questionable in the absence of a dose-response relationship.

Studies in mammalian cells *in vitro*:

- CHO cells

There was no increase in mutant frequency at concentrations associated with cytotoxicity (13% survival at the highest tested dose) in an HPRT assay using CHO cells without metabolic activation system. In contrast, increased chromosome aberrations were reported in the same study (Moore et al., 1989). In addition, in 1991, Moore *et al*, (only abstract available) compared the standard monolayer assay with a suspension adapted CHO assay that uses cell numbers comparable to that of the L5178Y mouse lymphoma assay. TMPTA was negative in both test systems in CHO cells.

- Mouse lymphoma L5178Y cells

In the first study (Anonymous, 1979), dose-related increase of mutant frequencies were reported in the absence of metabolic activation. The positive results were found with relative total growth (RTG) > 10%. The size of colonies was not reported to discriminate gene mutation or chromosomal aberration. In the presence of S9 activation, positive response was observed in 1 of the 3 independent experiments (the others were either equivocal or negative), only at the highest concentration associated with severe cytotoxicity (RTG = 4.8%). In summary, no consistent response was reported among the 3 experiments making the conclusion difficult.

In a second study, increased mutant frequency was observed with TMPTA only in the absence of metabolic activation at concentrations leading to cytotoxicity (RTG at 14.5% and 5% at the two highest concentrations, respectively). The size of colonies was not reported to discriminate gene mutation and chromosomal aberration (Cameron, 1991).

Similar results were also obtained by Dearfield and Moore (1989), though no metabolic activation system was used. One culture was used for mutation analysis and one for cytogenetics (chromosomal aberrations assay and cytochalasin B micronucleus analysis). A dose dependent increase in mutant frequency was obtained at doses showing about 50% cytotoxicity or more. Colony sizing indicated that TMPTA almost induced small colonies, suggesting a clastogenic mechanism. This was supported by increased aberrations and micronucleus frequencies.

- Human lymphocytes

Statistically significant and concentration-related increases in the frequency of cells with structural chromosomal aberrations were noted in two independent experiments, with and without metabolic activation. The positive response occurred at lower concentrations without metabolic activation (Anonymous, 2005). Without S9 mix, the increase in the frequency of cells with structural chromosomal aberrations was noted at 18.75 µg/mL (mitotic index = 55%) in the first experiment and at concentrations ≥ 9.38 µg/mL (mitotic index = 99%) in the second experiment. With S9 mix, the increase in the frequency of cells with structural chromosomal aberrations was noted at concentrations ≥ 37.5 µg/mL (mitotic index = 48%) in the first experiment and at concentrations of 28.13 and 50 µg/mL (no clear evidence of concentration-relationship; mitotic index = 113% and 63%, respectively) in the second experiment.

Conclusion of *in vitro* studies :

In summary, results from all *in vitro* studies showed that TMPTA induced chromosome aberrations in human lymphocytes and CHO cells and mutagenic responses (likely by a clastogenic mode of action) in L5178Y cells. The addition of metabolic activation decrease the genotoxic response suggesting an effet associated to TMPTA itself rather than its metabolites. The positive results were reported in the presence of cytotoxicity (of various degree).

GENETIC TOXICITY *IN VIVO*

In vivo cytogenicity:

Two *in vivo* micronucleus studies are available in mice, both reporting negative results.

The first study (Anonymous, 2006) was performed according to OECD 474 but presents some limitations: low number of animals analyzable per group and mainly the fact that there was no evidence of bone marrow exposure. Indeed, even if 2 deaths were reported at the highest dose in males (reason unknown), no systemic effect was found in females. PCE/NCE ratio was not altered and plasma levels of the test substance were not investigated. Furthermore, no kinetics data was available in mice to estimate the distribution profile of TMPTA after oral exposure. Therefore, from this study, the negative result is questionable since there is no adequate evidence of target tissue (bone marrow) exposure.

The second study (NTP, 2005) has been disregarded because it does not follow any guideline and no positive control was included to validate the protocol.

In the *in vivo* Comet assay, mice (6/group) were exposed to TMPTA in PEG 400 by slow intravenous (IV) bolus injection directly into the femoral vein via a surgical cannula (Anonymous, 2018). This study was also described and assess in the review of Kirkland and Fowler (2018). A first experiment was performed at 2.5, 5 and 10 mg/kg bw (based on an initial range-finding study showing clonic convulsion and twitching at 20 mg/kg bw) on two consecutive days. Only females were tested in the study considering that there was no

sex-difference in the range-finding study. Liver and bone marrow were sampled at necropsy, 30 minutes after the last administration. No increase of DNA damage was reported either in the liver and the bone marrow. Given the heterogeneity of the formulations, it was not possible to demonstrate exactly what the animals had been administered. Therefore, the laboratory decided to perform a new experiment. The second experiment consisted on the IV administration of TMPTA in PEG 400 at 5, 10 or 20 mg/kg bw on two consecutive days. The doses were selected based on a second range-finding study showing clinical effects (mainly clonic convulsion and hunched posture) and body weight loss at 30 mg/kg bw. Liver and bone marrow were sampled at necropsy, 30 minutes after the last administration. In this second experiment, there were no dose related increases in percentage of hedgehogs in the liver and bone marrow. In the liver, the mean tail intensity values for all treated groups were not significantly increased. According to the authors, the mean tail intensity values were significantly increased at 5 and 10 mg/kg bw in the bone marrow but not at 20 mg/kg bw.

Some limitations should be noted on this study:

First, the choice of the solvent is rather unusual. Due to its viscous properties and its anti-inflammatory properties, PEG 400 appears not a suitable solvent. The viscous properties of PEG 400 is not favourable to intravenous injection. In this context, and considering the soluble properties of TMPTA in organic solvents, it is not clear why a more common solvent had not been used (ex. CMC or corn oil). In the literature, PEG 400 is reported as well tolerated in different species and several routes, including IV route (Pandey *et al*., 2017; Gad *et al*., 2016; Healing *et al*., 2015; Thackaberry *et al*., 2010). However, publications report anti-inflammatory / anti-oxidant properties of PEG 400 as well as some protective effects when administered with other substances (Ackland *et al*., 2010; Juarez-Moreno *et al.*, 2015, Ma *et al.*, 2017; Hodoshima *et al.*, 2004; Klugman *et al.*, 1981). In particular, pegylation is used in pharmaceutical sector in order to improve the tolerability of medicine. Considering that, PEG 400 may reduce or affect the intrinsic toxicity of TMPTA. It can be hypothesized that PEG 400 may counteract the irritation induced by TMPTA that may contributed to DNA damage. In this context, it cannot be ruled out that using PEG 400 may mask/decrease the reactivity of TMPTA.

Secondly, according to OECD guideline 489, "*animals should be given daily treatments over a duration of 2 or more days (i.e. two or more treatments at approximately 24 hour intervals), and samples should be collected once at 2-6 h (or at the Tmax) after the last treatment*". In contrast, in the study, the samples were collected 30 min after the last treatment. Even if this short interval may be justified by the IV administration, there is no adequate kinetics study to confirm the relevance of this sampling time. For example, an immediate Tmax (< 30 minutes) can be expected after parenteral administration. In this context, the sampling time is not compliant with the OECD guideline since the Tmax was not checked.

Finally, according to the authors, the increased mean tail intensity values reported in bone marrow in the second experiment remained within the historical control. However, the reported historical vehicle controls are not considered relevant since they consist only on 5 animals exposed orally to CMC (and not PEG 400 administered by IV route as in the present study). In addition, it is noted that the tail intensity mean in the bone marrow (0.18) with PEG 400 is lower than that reported with these historical control with CMC as solvent (0.24-0.72). In this context, only comparison with the concurrent vehicle data is judged appropriate.

Additional remarks can be made on the interpretations of the results:

Inadequate results for achieved concentration and homogenicity were noted in the first experiment. Even if this experiment can not be used for concluding on mutagenicity of TMPTA, it does not indicate genotoxicity of TMPTA at the nominal concentrations tested.

The statistical significance of the result at 10 mg/kg bw in the second experiment seems questionable (mean tail intensity: 0.29 versus 0.18 in the control group). The statistical test used in this study (Anova) is probably not relevant since the variances are not homogenous. When using a non-parametric test (Kruskall-Wallis), no statistically significant increase was noted at the dose of 10 mg/kg bw. Only the increase of DNA damage at 5 mg/kg bw remains statistically significant.

In conclusion, DNA damages were increased in the bone marrow without a dose-response relationship in one of the two experiments performed. The Comet assay presents several biais for firmly concluding on genotoxicity of TMPTA.

10.6.2 Comparison with the CLP criteria

The classification in Category 1A *is based on positive evidence from human epidemiological studies.*

There is no evidence of germ cell mutagenicity of TMPTA from human epidemiological studies.
Thus, the substance should not be classified as Muta. Cat. 1A

The classification in Category 1B *is based on:*

– positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or

No *in vivo* heritable germ cell mutagenicity tests in mammals is available with TMPTA.

– positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or

A statistically significant increase of DNA damage was reported in bone marrow from the *in vivo* Comet assay. However, this test remains inconclusive due to the limitations reported above. There is no data allowing demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells.

– positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

There is no study available with TMPTA on germ cells of humans.

In conclusion, available data do not allow classifying the substance as Muta. Cat. 1B.

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.

TMPTA is clastogenic *in vitro* in various assays in mammalian cells. A statistically significant increase of DNA damage was reported in bone marrow from the *in vivo* Comet assay. However, this test remains inconclusive due to the limitations reported above.

Evaluation of hazard information:
Regarding positive findings, responses generated only at highly toxic/cytotoxic concentrations should be interpreted with caution, and the presence or absence of a dose-response relationship should be considered.

In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

In vitro, some Ames tests reported positive results in TA1535 in the presence of metabolic activation system (from rat or hamster liver). The positive results occurred in the absence of dose-response relationship. Consistent clastogenic effects were reported in mammalian cells in the presence of cytotoxicity of various degrees.

In vivo, the available micronucleus assays with TMPTA reported negative results. However, no final conclusion can be made from these studies since there was no evidence that the target tissue had been adequately reached or no positive control was included. DNA damages increased with a statistically significance in the bone marrow of mice in a Comet assay, but with no dose-response relationship. No increase of DNA damage was reported in the liver, which is one of the target organ of the carcinogenicity of TMPTA (see section 10.7 below).

In conclusion, the available data do not allow classifying the substance as Muta. Cat. 2

10.6.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on

- *in vitro* data: clastogenic effects reported at cytotoxic doses;
- *in vivo* data: negative or unconclusive data

The substance cannot be classified as a Germ cell mutagen according to CLP Regulation.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro data

The dossier submitter (DS) summarised four bacterial reverse mutation assays, four gene mutation and chromosome aberration assays in mammalian cells, and one chromosome aberration test in primary human lymphocytes.

Negative results were found with *S. typhimurium* (TA 98, TA 100, TA 1535, TA 1537) and *E. coli* (WP2 uvrA/pKM101) with and without metabolic activation. Positive results (increases between 1.6 and 4.8 fold) were reported for TA 1535 only in the presence of metabolic activation in two of the studies. The biological relevance of the findings was deemed questionable due to absence of a dose-response relationship.

In Chinese hamster ovary (CHO) cells, at concentrations up to 0.7 µg/mL without metabolic activation, TMPTA induced chromosome aberrations but no gene mutations. Cytotoxicity was observed at all doses tested (survival down to 72%, 22%, and 13% in low, mid, and high dose, respectively). Concentration-related increases in mutant frequencies were reported in mouse lymphoma cells in three studies without metabolic activation at cytotoxic concentrations of the test substance. The size of colonies was not reported in two of the studies to discriminate gene mutation or chromosomal aberration. Colony sizing in the third study indicated that TMPTA induced small colonies, suggesting a clastogenic mechanism.

In primary human lymphocytes, statistically significant and concentration-related increases in

the frequency of cells with structural chromosomal aberrations were noted in two independent experiments, with and without metabolic activation. Only the lowest concentration inducing a positive response in the second experiment was not cytotoxic (cytotoxicity in higher concentrations ranged from 26% to 100%). The positive response occurred at lower concentrations without metabolic activation, indicating a direct effect of the parent substance rather than its metabolites.

In vivo data

Two micronucleus studies in mice with negative results were summarised by the DS. Both studies had a number of limitations. In the first there was no evidence of bone marrow exposure, a low number of animals was used, and no measurement of plasma levels of the test substance was performed. The second study did not follow a guideline and no positive control was included.

In a comet assay in mice, DNA damages were increased in the bone marrow without a dose-response relationship in one of the two experiments performed. This study also had various limitations. The test substance was applied via i.v. using PEG 400 as a solvent. The DS noted that this is a rather unusual solvent for i.v. application due to its viscous and anti-inflammatory properties. No Tmax measurement was performed and samples were taken after 30 min of exposure, which contradicts the guideline *("samples should be collected once at 2-6 h (or at the Tmax) after the last treatment"*). Furthermore, according to the DS statistical methods and historical control data (HCD) used were not appropriate. The DS concluded that although a statistically significant increase in DNA damage was observed in this study, data remain inconclusive due to the presented limitations.

DS conclusion on classification

In conclusion, based on clastogenic effects *in vitro* observed only at cytotoxic concentrations and negative or inconclusive data from *in vivo* experiments the DS proposed **no classification** for germ cell mutagenicity.

Comments received during consultation

One Member State Competent Authority (MSCA), the study director of the Comet assay, two individuals, and one industry association commented on this hazard class. The MSCA concurred with the DS that the available data are not sufficiently robust to trigger classification. The industry commenter and both individuals supported no classification of TMPTA for germ cell mutagenicity but argued that the data are indeed sufficient to draw a firm conclusion. RAC notes that the commenting individuals are the authors of a review on the mutagenicity of TMPTA, and both were financially supported by the industry association in preparing their comments. The study director of the Comet assay responded to doubts expressed regarding reliability of the study raised by the DS.

Their main arguments were the following:

- In the first micronucleus test in mice, bone marrow exposure is likely due to TMPTA's physico-chemical properties.
- The number of animals analysed was sufficient according to the guideline in place at the

time of the study.

- The second micronucleus study was performed by the NTP, thus by a laboratory experienced in this type of assay. Therefore, a concurrent positive control was not necessary.
- In the Comet assay, the use of PEG 400 is not as unusual as considered by the DS. In fact, PEG 400 proved to be a suitable solvent for TMPTA and has been used in several studies in the performing laboratory.
- Systemic toxicity at a dose of 30 mg/kg bw (convulsions, hunched posture, body weight loss) in the first experiment indicate that any protective effect of the solvent was overcome at the top dose of 20 mg/kg bw (established as MTD by the study authors) in the second experiment. Furthermore, an anti-irritative effect of the solvent would not prevent a genotoxic effect.
- The sampling time was appropriate given i.v. application and is standard practice of the laboratory for i.v. studies. Furthermore, NTP data of five male rats showed that five minutes after bolus injection maximum blood levels were reached.
- Since there was no dose-response relationship in the positive responses in mouse bone marrow the biological relevance of these results is questionable regardless of statistical significance.
- The statistical method used is referenced in the OECD TG 489.

Assessment and comparison with the classification criteria

In vitro data

All bacterial mutation assays were performed according to OECD TG 471. In the first bacterial reverse mutation assay (AMES test) using *S. typhimurium* strains, negative results were observed up to a concentration of 500 µg/plate with and without metabolic activation. No cytotoxicity was reported for the incorporation assay. In a second AMES test using the same strains with doses up to 5000 µg/plate, positive results were observed only for TA 1535 with metabolic activation at doses from 500 µg/plate without a clear dose-response relationship and in absence of cytotoxicity. In the third AMES test with up to 10000 µg/plate again positive results were only observed for TA 1535 with metabolic activation without cytotoxicity. This strain was tested with up to 6667 µg/plate TMPTA and four different concentrations of S9 mix. There was no dose-response relationship observed in any of the settings. In the last bacterial reverse mutation assay (pre-incubation method) with *S. typhimurium* strains TA 98 und TA 100 and *E. coli* WP2 *uvrA*/pKM101 with concentrations up to 10000 mg/plate, slight cytotoxicity was observed in the highest dose with S9 mix. The test was negative for all tested strains.

All four gene mutation assays summarised in the CLH report were conducted using protocols similar to OECD TG 476. In the first assay with mouse lymphoma L5178Y TK+/- cells, positive results were reported in all three trials without metabolic activation at concentrations inducing cytotoxicity (19 to 33% relative growth). With metabolic activation, results were inconclusive: inconclusive results in the first trial due to contaminations, negative results in the second trial, and positive results in the third trial at the highest dose (4.8% relative growth). In a second

study using mouse lymphoma cells without metabolic activation, small colonies, micronuclei, and chromosomal aberrations were induced at concentrations up to 0.7 µg/mL also inducing cytotoxicity as evidenced by survival rates of 51 to 28%. In the third study, positive results were also obtained in mouse lymphoma cells without metabolic activation at cytotoxic concentrations (relative growth 14.5 and 5%). Results with metabolic activation were negative in this study. In a study with CHO cells without metabolic activation, results were negative for gene mutation and positive for chromosome aberrations, again at concentrations leading to cytotoxicity (survival rates 72 to 13%).

Statistically significant and concentration-related increases in the frequency of primary human lymphocytes with structural chromosomal aberrations were noted in two independent experiments, with and without metabolic activation in a study performed according to OECD TG 473. Single positive results were observed at concentrations that did not induce cytotoxicity. Cytotoxicity ranged from 26 to 100% in the other concentrations.

RAC concurs with the DS that TMPTA induced chromosome aberrations in human lymphocytes and CHO cells, and mutagenic responses in mouse lymphoma cells at concentrations producing various degrees of cytotoxicity. The decrease in the genotoxic response with metabolic activation suggests that the effect is associated to TMPTA itself rather than its metabolites. Results in bacterial tests are equivocal but negative for most tested strains.

In vivo data

Two micronucleus tests in mice yielded negative results. In the first study (NTP study), male and female mice were dermally exposed to the test substance 5 times per week for 14 or 28 weeks up to a nominal concentration of 12 mg/kg bw/d. Blood was collected from the retro orbital sinus and stained for analysis of micronuclei and normochromatic/polychromatic erythrocytes (NCE/PCE) ratio. No guideline was followed and no positive controls were included in this study. The second study was performed according to OECD TG 474 and under GLP conditions. The test substance was administered once orally via gavage at concentrations up 2000 mg/kg bw. Piloerection was the only systemic effect observed. Accidental deaths due to dosing errors occurred in one male each of the vehicle control and low dose group and in four females of the high dose group, reducing the numbers of animals used for statistical analysis of results.

In a mouse alkaline Comet assay similar to OECD TG 489, 6 female mice per dose were exposed to two doses of up to 20 mg/kg bw by i.v. application in PEG 400 in 24-hour intervals. Clinical signs included convulsions, rapid and/or gasping respiration, staggering, lethargy, and dark eyes in some animals at the two highest doses. No significant effects on body weight, clinical chemistry, macroscopic and microscopic findings were reported. Livers and bone marrow were examined, samples were collected 30 min after exposure. Negative results were reported for liver samples. In the first test, inadequate results for the achieved test substance concentrations were reported. Statistically significantly increases of mean tail intensity values in bone marrow were observed at 5 and 10 mg/kg bw in the second test. The DS questioned the statistical method used. In their analysis, only the results at 5 mg/kg bw remained statistically significant. Overall, no dose response was observed. Moreover, the study director concluded that TMPTA did not induce biologically relevant increases in tail intensity in the liver or bone marrow of female mice under the conditions employed.

Regarding the *in vivo* data, RAC concludes that despite some limitations, the overall results were negative. The sampling time in the Comet assay seems appropriate given i.v. application

of the test substance. PEG 400 as a solvent may be an unusual choice but it did not mask toxic effects. Moreover, no dose-response was observed and results were negative in the highest dose-group.

Conclusion on classification

RAC concurs with the DS that the available data does not allow for classification in category 1B or 2 for germ cell mutagenicity. The guidance on classification states: *"Regarding positive findings, responses generated only at highly toxic/cytotoxic concentrations should be interpreted with caution, and the presence or absence of a dose-response relationship should be considered. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied."* Overall, the *in vitro* and *in vivo* results were negative or compromised by cytotoxicity or a lack of a dose response relationship.

In addition, the guidance on application of CLP criteria states: "*Classification in Category 2 may be based on positive results of at least one in vivo valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells."* There is no such test available.

Thus, RAC concludes that **no classification for germ cell mutagenicity** is warranted.

10.7 Carcinogenicity

Table 9: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference** **Reliability**
Mouse (C3H/HeJ) male A group of 50 male mice received topical application of 50 mg of TMPTA to shaved area of the back, twice weekly for 80 weeks. A group of solvent control and positive control (0.05% benzo(a)pyrene in mineral oil) were also maintained along with no-treatment control group in the study. Parameters evaluated included clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations.	TMPTA Purity not stated 50 mg (nominal, per mouse per application) Vehicle: paraffin oil No information if the application was occlusive or not Exposure: 80 weeks (Twice a week)	Non-carcinogenic effects: ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst Neoplastic effects: no effects	Anonymous (1982) 3 (not reliable)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference** **Reliability**
Mouse (Tg.AC hemizygous) male/female, 15/sex/group Application on the backs of male and female Tg.AC mice five times per week for 6 months. Animals painted with acetone alone served as the control groups. Tissues from 15 sites were examined for every animal.	TMPTA Purity = 80% 0, 0.75, 1.5, 3, 6, 12 mg/kg bw/day (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 6 months (5 days per week)	**Non-neoplastic effects:** effects on liver, lung, heart, kidney weight; hyperplasia, hyperkeratosis, chronic active inflammation; hematopoietic cell proliferation and myelodysplasia ➔ NOAEL for non-neoplastic effects = 1.5 mg/kg bw/day **Neoplastic effects**: Forestomach squamous cell papilloma (27-33-27-13-33-60%) in females and squamous cell papilloma at the site of application (0-0-0-13-80-87% in males and 0-0-0-7-73-100% in females) ➔ NOAEL for carcinogenicity = 3 mg/kg bw/day	NTP (2005) 2 (reliable with restrictions)
F344/N rats male/female; 65/sex/group Equivalent or similar to OECD Guideline 451	TMPTA Purity > 78% 0.3, 1.0, 3.0 mg/kg bw/day (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 104 to 105 weeks – dermal application (5 times per week) Interim evaluations performed after 2, 13 and 52 weeks	**Non-neoplastic effects:** epidermal hyperplasia, hyperkeratosis, chronic inflammation, hyperplasia in the adrenal medulla. NOAEL for local effect < 0.3 mg/kg bw/day for female rats. NOAEL for local effect = 0.3 mg/kg bw/day for male rats. NOAEL non-neoplastic systemic effect = 3 mg/kg bw/day (no effect in rats) **Neoplastic effects:** malignant mesothelioma (0-4-4-10%) in male rats NOAEL carcinogenicity = 1.0 mg/kg bw/day for male rats NOAEL carcinogenicity = 3.0 mg/kg bw/day for female rats	NTP (2012) 2 (reliable with restrictions)
B6C3F1 mice male/female; 65/sex/group Equivalent or similar to OECD Guideline 451	TMPTA Purity > 78% 0.3, 1.0, 3.0 mg/kg bw/day (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 105 to 106 weeks – dermal application (5 times per week)	**Non-neoplastic effects:** epidermal hyperplasia, hyperkeratosis, chronic inflammation, hyperplasia in the adrenal medulla. NOAEL for local effect = 0.3 mg/kg bw/day for mice. NOAEL non-neoplastic systemic effect = 1 mg/kg bw/day (hyperplasia in the adrenal medulla in male mice) NOAEL non-neoplastic systemic effect = 3 mg/kg bw/day (no effect female mice) **Neoplastic effects:** hepatoblastoma (0-8-0-6%), hepatocholangiocarcinoma (0-0-2-4%) and uterine stromal polyps or stromal sarcoma (combined) (0-2-4-12%) in female mice	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference Reliability
	Interim evaluations performed after 2, 13 and 52 weeks	NOAEL carcinogenicity = 0.3 mg/kg bw/day for female mice NOAEL carcinogenicity = 3.0 mg/kg bw/day for male mice	

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

Table 10: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	Strength of evidence (NTP)*	MoA and relevance to humans
Tg. AC hemizygous mice	Squamous cell papilloma	Yes	Yes	No conclusion	Both sexes	Severe dermal reactions	Dermal	-	Transgenic animals
	Forestomach squamous cell papilloma		No	No	Single sexe (female)	No			
B6C3F1 mice	Stromal polyps or stromal sarcoma (combined)	Yes	No	No conclusion	Single sexe (female)	No	Dermal	Some	Assumed
	Hepatoblastoma and hepatocholangiocarcinoma		Yes: already malignant			No		Some	
F344/N rats	Malignant mesothelioma	No	Yes: already malignant	No conclusion	Single sexe (male)	No	Dermal	Equivocal	Assumed

* ***Some Evidence of Carcinogenic Activity*** is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

Equivocal Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

The NTP conducted an assay with TMPTA dermally applied to genetically modified strain of mouse (NTP, 2005). The Tg. AC hemizygous mice used contains an oncogene, v-Ha-ras transgene, so this model is genetically initiated and sensitive to dermal tumour promoters. Tg. AC hemizygous mice were administered 0, 0.75, 1.5, 3, 6 or 12 mg TMPTA/kg bw in acetone 5 days per week for 28 weeks. A group of positive control received dermal applications of 12-O-tetradecanoylphorbol-13-acetate 3 days per week for 28 weeks. Survival and mean body weights of dose groups were similar to those of the vehicle controls. There were some effects on organ weights (liver, lung, heart and kidney) without corresponding histopathological findings. Increased incidences of minimal to moderate (mostly mild) hyperplasia of the epidermis (from 3 mg/kg bw), hyperkeratosis (from 3 mg/kg bw), and chronic active inflammation (from 6 mg/kg bw) also occurred at the site of application. A hematopoietic cell proliferation and myelodysplasia occurred in both male and female mice at the highest dose. These changes may be attributed to dermal inflammation. Mice had significantly increased incidences and multiplicity of squamous cell papillomas of the skin at the site of dermal application from 6 mg/kg bw (0%, 0%, 0%, 13%, 80%, 87% in males and 0%, 0%, 0%, 7%, 73%,

100% in females, for each dose, respectively). Squamous cell carcinomas occurred at the site of application in one female at 1.5, 6 and 12 mg/kg bw. These carcinomas appeared to arise within papilloma. Thus they were considered related to treatment and possibly the result of malignant conversion of papilloma. Increased incidences of forestomach squamous cell papilloma in female mice at 12 mg/kg bw (27%, 33%, 27%, 13%, 33%, 60% for each dose, respectively) may have been related to chemical administration since the incidence is higher than the common spontaneous rate (10-25% in hemizygous females (Mahler *et al.* 1998) and Eastin *et al.* (2001) cited in NTP (2005)).

In a standard carcinogenicity study performed by NTP in 2012, mice or rats were dermally exposed to 0, 0.3, 1.0, and 3.0 mg TMPTA/kg bw in acetone for 2 years (5 days per week). Interim kills of 5 animals per sex and group were performed after 2, 13, and 52 weeks for examination of skin tissue.

Historical Database: as a significant factor affecting the background incidence of neoplasms at a variety of sites is diet, the NTP historical database mentioned below contains all studies that use the NTP actual diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. For the current study, the incidences for dermal studies with all vehicles were combined because the historical database does not include any other dermal studies with acetone as the vehicle; these incidences and the overall incidences for all routes of administration were used for comparison.

Non-neoplastic findings: Survival and body weight gain were unaffected by the test substance. Rats and mice of the mid and high dose groups showed increased incidences of epidermal hyperplasia, hyperkeratosis, and signs of chronic inflammation (mice). Hyperkeratosis was also reported at the lowest tested dose in female rats. Despite the increase of epidermal hyperplasia characteristic of tumour promotion, no increase in skin tumors compared to control animals could be detected.

Table 11: Incidence of nonneoplasic lesions of the skin at the site of application in core study rats in the 2-year dermal study of trimethylolpropane triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Number Examined Microscopically	50	49	50	50
Epidermis, Hyperplasia[a]	1 (1.0)[b]	0	12** (1.0)	28** (1.1)
Hyperkeratosis	2 (1.0)	4 (1.0)	33** (1.0)	49** (1.0)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	0	4 (1.3)	11** (1.0)	25** (1.0)
Hyperkeratosis	0	11** (1.0)	42** (1.0)	50** (1.0)

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table 12: Incidence of nonneoplasic lesions of the skin at the site of application in core study mice in the 2-year dermal study of trimethylolpropane triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia[a]	10 (1.3)[b]	7 (1.9)	15 (1.2)	44** (1.7)
Hyperplasia, Melanocyte	0	0	0	19** (1.1)
Inflammation, Chronic	13 (1.2)	17 (1.1)	26** (1.1)	43** (1.3)
Female				
Number Examined Microscopically	50	50	50	50
Epidermis, Hyperplasia	7 (1.9)	7 (1.6)	15* (1.5)	34** (1.7)
Hyperplasia, Melanocyte	1 (1.0)	1 (4.0)	3 (1.7)	33** (1.3)
Inflammation, Chronic	37 (1.1)	36 (1.2)	43 (1.2)	48** (1.5)
Ulcer	0	0	3 (3.3)	3 (3.3)
Inflammation, Acute	1 (2.0)	1 (3.0)	2 (2.5)	4 (1.5)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

There was a statistically significant increase of hyperplasia in the adrenal medulla (1/49, 4/49, 3/46, 10/50) in male mice at the highest tested dose, with positive trend. The incidence at 3 mg/kg bw/day also exceeded the historical range (0-8%) in concurrent NTP studies by all routes. In addition, there was a significantly increased incidence of mineralization in the glandular stomach (1/48, 3/49, 2/44, 8/49). This effect was considered sporadic and most likely unrelated to TMPTA administration by the NTP because it is a common background lesion. In female mice, there was a significant increase of the incidence of eosinophilic focus and Kupffer cell pigmentation but the relationship with TMPTA administration is uncertain.

Carcinogenic findings:

No test-substance related increase in neoplastic lesions was found in male mice and female rats. In male mice, there was a significant increase in the incidence of alveolar/bronchiolar adenoma at 1 mg/kg bw; however, the alveolar/bronchiolar carcinoma decreased in this group (see Table 13). This was not considered treatment-related due to the absence of a significant positive trend and the incidences were within the historical control ranges.

Table 13: Summary of the Incidence of Alveolar/bronchiolar adenoma and carcinoma in Male Mice in the 2-Year Dermal Study of Trimethylolpropane Triacrylate

	Vehicle control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Alveolar/bronchiolar adenoma	1/50 (2%)	6/50 (12%)	10/50 (20%)	4/50 (8%)
Alveolar/bronchiolar carcinoma	12/50 (24%)	11/50 (22%)	3/50 (6%)	10/50 (10%)

In male rats, there was a statistically significant increase in the incidence of malignant mesothelioma (overall rate: 0%, 4%, 4%, 10% at 0, 0.3, 1, 3 mg/kg) at 3 mg/kg bw/day, with a significant positive trend. The incidence at the highest dose exceeded historical control ranges for dermal studies (all vehicles) and for all routes of administration (0-8%). In all cases, they arose from the tunics around the testes. Maronpot *et al.* (2009) reported that tunica vaginalis mesothelioma induction is a male F344 rat-specific event associated with a high background incidence of Leydig-cell tumors and are thus likely to be irrelevant in human risk

assessment. It should be noted that Maronpot *et al* (2009) concluded on human relevance on the basis of old articles (1992-1997) stating rarity of human Leydig cell tumors. Owing to knowkedge gained in the two last decades, the evaluation has changed to-day and needs updating. Furthermore, the incidence of interstitial cell adenoma in testes were not increased in treated groups (54%, 34%, 54% and 56% for each groups) in the NTP (2012) study, refuting the Maronpot *et al* (2009) conclusion. Because the incidence of malignant mesothelioma in the high dose group was only one tumor outside of the historical control range, this finding was considered by the NTP to be an equivocal evidence of carcinogenic activity of trimethylolpropane triacrylate in male rats.

Table 14: Incidence of malignant mesothelioma in male rats in the 2-year dermal study of trimethylolpropane triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Overall rate[a,b]	0/50 (0%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rate[c]	0.0%	5.7%	4.9%	11.8%
Terminal rate[d]	0/23 (0%)	1/18 (6%)	1/28 (4%)	1/23 (4%)
First incidence (days)	—[f]	529	728	591
Poly-3 test[e]	P=0.024	P=0.201	P=0.231	P=0.031

[a] Number of animals with malignant mesothelioma per number necropsied

[b] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 8/250 (3.2% ± 3.4%), range 0%-8%; all routes: 40/1,249 (3.2% ± 2.8%), range 0%-8%

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[f] Not applicable; no neoplasms in animal group

In female mice, although not significant, there was an increase of incidences of hepatoblastoma (0%, 8%, 0%, 6% for control, low, mid, and high dose) and hepatocholangiocarcinoma (0%, 0%, 2%, 4%) which exceeded historical control ranges. The historical control ranges for hepatoblastoma are low (0-2%) while hepatocholangiocarcinoma has not been seen in historical controls. Based on the rarity of these neoplasms in female mice and their absence in the concurrent vehicle controls, these tumours are considered to be biologically significant and related to treatment. NTP concluded that these findings constitute some evidence of carcinogenic activity. There was also a small but significant positive trend in the incidence of hepatocellular carcinoma in female mice.

Table 15: Incidences of neoplasms of the liver in female mice in the 2-year dermal studyof trimethylolpropane triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Number Examined Microscopically	50	50	50	50
Hepatoblastoma, Multiple[a]	0	1	0	0
Hepatoblastoma, (includes multiple)[b]	0	4	0	3
Hepatocholangiocarcinoma[c]	0	0	1	2
Hepatocellular Carcinoma, Multiple	3	3	5	2
Hepatocellular Carcinoma (includes multiple)[d]				
Overall rate[e]	12/50 (24%)	13/50 (26%)	10/50 (20%)	19/50 (38%)
Adjusted rate[f]	25.4%	28.4%	22.7%	41.3%
Terminal rate[g]	10/39 (26%)	6/31 (19%)	7/30 (23%)	12/30 (40%)
First incidence (days)	638	513	440	599
Poly-3 test[h]	P=0.045	P=0.461	P=0.479N	P=0.076

[a] Number of animals with neoplasm

[b] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 2/250 (0.8% ± 1.1%), range: 0%-2%; all routes: 4/1,195 (0.3% ± 0.8%), range: 0%-2%

[c] Historical incidence for 2-year dermal studies: 0/250; all routes: 0/1,195

[d] Historical incidence for 2-year dermal studies: 63/250 (25.2% ± 15.5%), range: 6%-46%; all routes: 144/1,195 (12.1% ± 10.8%), range: 0%-46%

[e] Number of animals with neoplasm per number of animals with liver examined microscopically

[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[g] Observed incidence at terminal kill

[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by N.

The incidences of uterine stromal polyps or stromal sarcoma (combined) were statistically significantly increased in the 3 mg/kg mice group and exceeded NTP historical control data for dermal studies (all vehicles) and for all routes of administration (0-8%). The incidences of polyps or stromal sarcoma (combined) were 0%, 2%, 4%, and 12% in control, low, mid, and high dose female mice. This result is mainly driven by the increase of stromal polyps since only one sarcoma was found at 3 mg/kg bw/day. However, it should be noted that uterine sarcoma is a rare finding in dermal studies (historical incidence: 0/250). In a publication by Davis (2012), a range of 0-14.3% is reported in B6C3F1/N female mice for the incidence of benign stromal polyps. These historical control data were obtained from 29 carcinogenicity studies terminated between 1988 and 1998. In these studies, the diet (Altromin 1321) was different from that used in the NTP study (NTP-2000). Since the NTP historical database is consistent in term of diet and contains contemporary studies (histopathological findings completed within the 5-years before the study performed with TMPTA), it is more relevant to compare the incidence of uterine polyps obtained after TMPTA exposure with these historical control data. Although there are some differences in the physiopathology of uterine polyps between women (that develop from both endometrial and stromal components and are hormono-sensitive) and rodents (that develop from stromal components only and do not appear to be hormonally sensitive), these tumours can be an indicator of carcinogenesis with an unknown mechanism of action leading to effects occurring in other human target tissues. In this context, the increased incidence of uterine stroma polyps and stromal sarcoma are judged biologically relevant. Finally, NTP concluded that the increased incidence of uterine stromal polyps provided some evidence of carcinogenic activity.

Table 16: Incidence of neoplasms of the uterus in female mice in the 2-year dermal study of trimethylolpropane triacrylate

	Vehicle Control	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Number Necropsied	50	50	50	50
Stromal Polyp[a]				
Overall rate[b]	0/50 (0%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate[c]	0.0%	2.3%	4.7%	11.1%
Terminal rate[d]	0/39 (0%)	1/31 (3%)	2/30 (7%)	4/30 (13%)
First incidence (days)	—[f]	729 (T)	729 (T)	409
Poly-3 test[e]	P=0.008	P=0.486	P=0.219	P=0.027
Stromal Sarcoma[g]	0	0	0	1
Stromal Polyp or Stromal Sarcoma[h]				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	2.3%	4.7%	13.3%
Terminal rate	0/39 (0%)	1/31 (3%)	2/30 (7%)	4/30 (13%)
First incidence (days)	—	729 (T)	729 (T)	409
Poly-3 test	P=0.002	P=0.486	P=0.219	P=0.014

(T) Terminal kill

[a] Historical incidence for 2-year dermal study vehicle controls (all vehicles) (mean ± standard deviation): 5/250 (2.0% ± 2.5%), range 0%-6%; all routes: 24/1,198 (2.0% ± 2.2%), range 0%–8%

[b] Number of animals with neoplasm per number of animals necropsied

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[f] Not applicable; no neoplasms in animal group

[g] Historical incidence for 2-year dermal studies: 0/250; all routes: 2/1,198 (0.2% ± 0.6%), range 0%-2%

[h] Historical incidence for 2-year dermal studies: 5/250 (2.0% ± 2.5%), range 0%-6%; all routes: 26/1,198 (2.2% ± 2.2%), range 0%-8%

Finally another study assessing the carcinogenic potential of TMPTA in mice is available (Anonymous, 1982). This study is inadequate and should be disregarded due to several limitations (only males, low frequency of application, unique concentration tested, lack of information on purity, poorly described). C3H/HeJ male mice received topical application of 50 mg of the test substance (5 % in white mineral oil) to shaved area of the back, twice weekly for 80 weeks. A group of solvent control and positive control (0.05% benzo(a) pyrene in mineral oil) were also maintained along with no-treatment control group in the study. Parameters evaluated included clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations. The gross observations made at necropsy were dark red lesions in lungs, liver tumors, kidney haemorrhages, enlarged spleen, skin ulcers, flaky skin, enlarged and grey lymph nodes, haemorrhages in stomach, grey or yellow spots in adrenals. Non-neoplastic histopathological lesions included ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst. No skin tumors were found in treated animals.

Conclusion:

TMPTA produced skin and forestomach neoplasms in Tg.AC hemizygous mouse model (NTP, 2005). Analysis of Tg.AC hemizygous mouse studies showed 77% accuracy in identifying known human carcinogens (Pritchard *et al.* 2003 cited in NTP 2012). Although this type of assay cannot be considered as a

definitive proof of carcinogenicity, the findings suggest that TMPTA is likely to be carcinogenic in a 2-year bioassay.

In the 2-year carcinogenicity study (NTP, 2012), carcinogenic effects were reported in female mice (stromal polyps, hepatoblastoma and hepatocholangiocarcinoma) and in male rats (malignant mesothelioma). TMPTA did not increase tumor formation at the site of application in the skin, contrary to the results in the Tg.AC mouse assay (NTP 2005). This discrepancy could be due to increased sensitivity of the Tg.AC hemizygous mouse skin to tumor promoters. The Tg.AC hemizygous mouse contains an oncogene, v-Ha-ras transgene, so this model is genetically initiated and sensitive to dermal tumor promoters. In addition, it should be noted that skin tumours mainly occurred from 6 mg/kg bw/day although the 2-year carcinogenicity study was performed at doses up to 3 mg/kg bw/day. In conclusion, TMPTA presents carcinogenic effects in transgenic mice of both sexes and in female mice and male rats in a 2-year study.

10.7.2 Comparison with the CLP criteria

Table 17: Comparison of toxicological results with CLP criteria

Toxicological results	CLP criteria
No data human data is available regarding carcinogenicity of TMPTA. Thus a classification as Carc. 1A is not appropriate for TMPTA.	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence
Strenght of evidence: Some evidence of carcinogenic effect (with both malignant and benign tumours) was reported by the NTP in female mice. Equivocal evidence of carcinogenic effect (malignant tumours) was reported by the NTP in male rats. No evidence of carcinogenic effect was observed in female rats and male mice. Tumours (benign) were also reported in transgenic mice. Tumour type and background incidence: Female mice: - Uterine stromal polyps or stromal sarcoma (combined) were significantly increased and exceed HCD (historical control data). The increase is mainly driven by the increase in stromal polyps; - Incidences of hepatoblastoma and hepatocholangiocarcinoma were increased (not significantly) but exceeded HCD. Male rats: - Incidence of malignant mesothelioma from tunica around testis were significantly increased and exceed HCD by one tumor.	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification is based on strength of evidence together with additional considerations: — animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). Carcinogenicity in experimental animals — sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of

Transgenic mice: - Significant increased incidences and multiplicity of squamous cell papillomas of the skin in both sexes; - Increased incidence of forestomach papilloma (not significant) in females. <u>Multi-site responses:</u> TMPTA induced tumours at 2 sites (uterus and liver) in female mice but only at one site (mesothelioma) in male rats. In transgenic animals, tumours occurred at one site in males (skin) and 2 sites in females (skin and forestomach). <u>Progression of lesions to malignancy:</u> Malignant tumours were already found in liver and from the tunics around testes (mesothelioma). <u>Reduced tumour latency:</u> Female mice and male rats: No conclusion possible. Transgenic animals: No conclusion possible for squamous cell papillomas, not found in control animals. No reduced latency for forestomach cell papilloma. <u>Whether responses are in single or both sexes</u>: Tumours were reported in one sexe (female) of mice and one sexe (male) of rats. Tumours were reported in both sexes of transgenic mice. <u>Whether responses are in a single species or several species:</u> Tumours were reported in rats and mice. <u>Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:</u> No information. <u>Routes of exposure:</u> The available carcinogenicity studies were performed by dermal route. There is no data for other routes. <u>Comparison of absorption, distribution, metabolism and excretion between test animals and humans:</u> Based on *in vitro* kinetics data in humans and *in vivo* kinetics data in both rats and mice, dermal absorption of TMPTA seems to be the lowest in humans (< 1%) and the highest in mice (75%). However, some cautions	tumour or age at onset, or when there are strong findings of tumours at multiple sites. The placing of a substance in Category 2 (suspected human carcinogens) 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. Carcinogenicity in experimental animals — limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. Additional considerations: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of

should be taken with the results obtained from the *in vitro* study on human skin since only one high concentration was tested. The possibility of a confounding effect of excessive toxicity at test doses: No significant differences in survival were observed between any groups. Skin reactions (hyperplasia, hyperkeratosis, chronic inflammation) were observed in both rats and mice. But there is no reason to link these effects to the occurrence of the tumours observed in "standard animals". In contrast, squamous cell papilloma in skin could be related to dermal irritation reported in transgenic mice. Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity: At this time, the carcinogenic mode of action is unknown. Liver tumours and mesothelioma are considered relevant to humans. Some differences in the physiopathology of uterine polyps between women and rodents may question the relevance of this finding for humans. Relevance of tumours reported in transgenic animals to humans may be questionable considering the high sensivitity of transgenic animals. Consideration of mutagenicity: TMPTA is a clastogenic agent based on *in vitro* studies. *In vivo*, no effect was reported in micronucleus assays, without evidence that the bone marrow was actually reached. In an *in vivo* Comet assay performed in mice by intravenous route, some increased DNA damages were reported in the bone marrow. No increase of DNA damage was reported in the liver, which is one of the target organs of TMPTA carcinogenicity. Thus, it is not expected that the carcinogenic effects are linked to a genotoxic mechanism. Conclusion: Malignant tumours (mesothelioma) are reported in male rats and female mice (liver tumours).	excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

Benign tumours (uterine polyps) are reported in female mice. Benign tumours (skin and forestomach) are reported in transgenic mice. Based on these observation, sufficient evidence could be reached from animal studies. However, the evidence seems not strong enough to propose a classification as Category 1B based on the following considerations: - Among the reported tumours: o Common target organ for carcinogenicity was not identified between sexe or species; o The increase of hepatoblastoma is not dose-related; o Even if default assumption in CLH is finding in animals are relevant to human, some differences in the physiopathology of uterine polyps between women and rodents may question the relevance of this finding for humans; o Relevance of tumours reported in transgenic animals to humans may be questionable considering the high sensivity of transgenic animals. - Differences of dermal absorption between rodents and humans are expected; - No mutagenic effects reported in the liver in the *in vivo* Comet assay. - The target organs identified in the carcinogenicity studies are not identified as such in the available repeated-dose toxicity studies of shorter duration by dermal route. In this context, there is limited evidence of carcinogenicity according to CLP guidance thus a classification as category 2 carcinogen is proposed for TMPTA.	

10.7.3 Conclusion on classification and labelling for carcinogenicity

Malignant and benign tumours were reported either in male rats (mesothelioma), in female mice (hepatoblastoma, hepatocholangiocarcinoma, uterine polyps) or in transgenic mice (squamous cell papilloma). Based on considerations related to the type/occurrence of tumours and to absorption, relevance

to humans or mode of action, the evidence seems not strong enough to propose a classification as Category 1B. In this context, a classification as category 2 carcinogen – H351 is proposed for TMPTA according to CLP regulation.

By the way, based on these data, the IARC in 2018 classified TMPTA as "possibly carcinogenic to humans" (Group 2B), based on "sufficient evidence" of carcinogenicity in experimental animals and no data in humans (IARC, 2018).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS summarised three carcinogenicity studies in mice and rats with dermal application, two of which did not follow a guideline.

One of these studies used only one dose and sex (male), and a non-standard dosing regimen (twice a week for 80 weeks) and reported no neoplastic lesions. Non-neoplastic effects in this study consisted of ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst.

The second non-guideline study was performed in Tg.AC hemizygous mice that contain the v-Ha-ras oncogene making them sensitive to dermal tumour promoters. Mice (15/sex/dose) were applied TMPTA in acetone for six months and five days a week up to a nominal concentration of 12 mg/kg bw/d. Squamous cell papilloma of the forestomach above control incidence were observed in females of the highest dose group and skin squamous cell papilloma at the site of application starting from 3 mg/kg bw/d in males and females (statistically significant from 6 mg/kg bw/d). Squamous cell carcinomas occurred at the site of application in one female each at 1.5, 6 and 12 mg/kg bw/d. Non-neoplastic findings included effects on organ weights (liver, lung, heart, kidney) without histopathological correspondence, epidermal hyperplasia and hyperkeratosis from 3 mg/kg bw/d, and chronic inflammation at the site of application from 6 mg/kg bw/d. Survival and body weight gain remained unaffected.

One guideline study (OECD TG 451) was performed in rats and mice. Both species were applied nominal doses of 0, 0.3, 1.0 or 3.0 mg/kg bw/d of TMPTA in acetone five times a week for two years. Groups consisted of 65 animals per sex and dose. Non-neoplastic effects comprised epidermal hyperplasia, hyperkeratosis, and chronic inflammation starting from the lowest dose in female rats and from the mid dose in other animals. Additionally, hyperplasia of the adrenal medulla was observed in male mice of the highest dose group. Neoplastic findings slightly above corresponding historical control ranges were reported in male rats (malignant mesothelioma of the *tunica vaginalis*) and in female mice (hepatoblastoma and -cholangiosarcoma as well as uterine stromal polyps or stromal sarcoma). No treatment related neoplastic lesions were observed in female rats and male mice.

The DS also summarised five dermal repeated dose toxicity studies in B6C3F1 mice, F344 rats, and NZW rabbits. In rabbits, at a nominal dose of 500 mg/kg bw/d of the undiluted substance applied dermally for two weeks (five days per week) no local effects were observed. Systemic

effects could not be assessed due to a lack of information on incidences and severity of symptoms. In mice and rats, TMPTA in acetone consistently induced epidermal hyperplasia and degeneration, hyperkeratosis, hyperplasia of sebaceous glands, and chronic inflammation of the dermis at nominal doses up to 200 mg/kg bw/d in 16-day studies and up to 12 mg/kg bw/d in 14-week studies. No systemic effects were reported in any of these studies.

The DS concluded that TMPTA induced carcinogenic responses in transgenic mice of both sexes in a non-guideline study and in female mice and male rats in a 2-year study. Based on malignant tumours reported in male rats (mesothelioma) and female mice (liver tumours), benign tumours (uterine polyps) in female mice, and benign tumours (skin and forestomach) in transgenic mice they inferred that sufficient evidence for carcinogenicity of the substance was available from animal studies. However, the evidence is not strong enough to propose a classification as Category 1B, since no common target organ for carcinogenicity was identified, the increase of hepatoblastoma was not dose-related, there are differences in the physiopathology of uterine polyps between women and rodents, and relevance of findings in transgenic animals is questionable. Therefore, the DS proposed a classification as **Carc. 2; H351**.

Comments received during consultation

One MSCA, one company/manufacturer and one industry association commented on this hazard class. The MSCA supported the proposed Carc. 2 classification based on an increased incidence of malignant and/or benign tumours in female mice and male rats. Industry commenters did not support classification for carcinogenicity based on the following main arguments:

- Skin papillomas in transgenic mice were likely to have been induced by irritation that was also promoted by the choice of vehicle (acetone).
- Since no skin papillomas were observed in the other studies, this tumour type is specific to the transgenic strain used.
- Male F-344/N *tunica vaginalis* mesothelioma incidence marginally exceeded the HCD, but this is a strain and sex-specific lesion, considered of no relevance to man.
- Liver tumours in female mice exceeded the HCD, but both are often associated with liver adenomas and carcinomas, tumours characteristic to this strain.
- Mouse uterine stromal polyps were increased but are normal age-related benign findings. A single uterine stromal sarcoma at the top dose was within the HCD range, and a single uterine sarcoma was also reported in a control animal.
- No plausible mode of action relevant for humans has been established for any of the tumour types observed.

Relevance of tumour types, choice of vehicle, and a lack of data for establishing a mode of action are all discussed in the CLH report. The DS in their response to the comments concluded that whilst the evidence of carcinogenicity of TMPTA is not sufficient to propose a classification as Carc. 1B; the proposed classification as Carc. 2 is based on a weight of evidence approach considering all tumours reported in mice and rats.

Assessment and comparison with the classification criteria

Repeated Dose Toxicity and Absorption Data

There are no human data on the carcinogenicity of TMPTA available. Dermal repeated dose toxicity studies consistently resulted in irritative effects on the skin of rats and mice when the substance was applied in acetone as a solvent. Absorption studies in rodents showed that dermal absorption is higher in mice than in rats and is inversely proportional to the applied dose. In an *in vitro* percutaneous absorption assay in human skin, with 9.1 mg/cm^2 neat substance absorption rate was 0.6%. No lower doses were tested, therefore comparison with rodent skin absorption is difficult.

Carcinogenicity Data

Carcinogenicity studies with dermal application were performed in rats and mice and are summarised in the table below.

Table: *Dermal carcinogenicity studies with TMPTA (modified from table 9 of the CLH report)*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**
Mice (C3H/HeJ) one treatment group: 50 males controls: solvent, positive (0.05% benzo(a)pyrene in mineral oil), no-treatment no guideline	TMPTA Purity not stated 50 mg (nominal, per mouse per application) Vehicle: paraffin oil No information if the application was occlusive or not Exposure: 80 weeks (twice a week)	Parameters evaluated included: clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations. **Non-neoplastic effects**: ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst **Neoplastic effects:** none
Mice (Tg.AC hemizygous) 15/sex/group control: solvent no guideline	TMPTA Purity = 80% 0, 0.75, 1.5, 3, 6, 12 mg/kg bw/d (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 6 months (5 days per week)	Tissues from 15 sites evaluated for each animal **Non-neoplastic effects:** effects on liver, lung, heart, kidney weight; hyperplasia, hyperkeratosis, chronic active inflammation; hematopoietic cell proliferation and myelodysplasia NOAEL: 1.5 mg/kg bw/d **Neoplastic effects**: Forestomach squamous cell papilloma (females) squamous cell papilloma at the site of application (males and females) NOAEL: 3 mg/kg bw/d

Rats (F344/N) 65/sex/group Equivalent or similar to OECD TG 451	TMPTA Purity > 78% 0, 0.3, 1.0, 3.0 mg/kg bw/d (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 104 to 105 weeks (5 times per week) Interim evaluations: after 2, 13, and 52 weeks	**Non-neoplastic effects:** epidermal hyperplasia, hyperkeratosis, chronic inflammation NOAEL (females): 0 mg/kg bw/d NOAEL (males): 0.3 mg/kg bw/d **Neoplastic effects:** malignant mesothelioma (males) NOAEL: 1.0 mg/kg bw/d
Mice (B6C3F1) 65/sex/group Equivalent or similar to OECD TG 451	TMPTA Purity > 78% 0, 0.3, 1.0, 3.0 mg/kg bw/d (nominal conc.) Vehicle: acetone No information if the application was occlusive or not Exposure: 105 to 106 weeks (5 times per week) Interim evaluations: after 2, 13 and 52 weeks	**Non-neoplastic effects:** epidermal hyperplasia, hyperkeratosis, chronic inflammation; hyperplasia in the adrenal medulla (males). NOAEL: 0.3 mg/kg bw/d NOAEL (hyperplasia of adrenal medulla): 1 mg/kg bw/d **Neoplastic effects:** hepatoblastoma, hepatocholangiocarcinoma, uterine stromal polyps, stromal sarcoma (females) NOAEL: 0.3 mg/kg bw/d

No effects on survival or body weight gain as compared to controls were observed in any of the studies. Due to the non-standard dosing regimen and the usage of one dose and sex only, RAC considers the first study not suitable for classification purposes.

In the study with transgenic mice, skin squamous cell papilloma were observed only in combination with chronic active inflammation of the skin. Skin squamous carcinoma occurred only in single incidences at 1.5, 6, and 12 mg/kg bw/d but not at 3 mg/kg bw/d. The genetic predisposition to skin lesions of this strain makes interpretation of results with regards to human relevance difficult. Furthermore, no such tumours were observed in the standard carcinogenicity study despite inflammation at the application site. Forestomach papilloma were not accompanied by inflammation in this tissue but incidences were not dose-dependent and reached statistical significance only in high dose females (9/15 vs 4/15 in controls).

RAC considers the guideline study in rats and mice to be the key study. Incidences and HCD for neoplastic lesions observed in this study are summarised in the table below.

Table: *Neoplastic findings in 2-year dermal carcinogenicity study in mice and rats (data extracted from tables 14-16 of the CLH report, HCD as provided in the CLH report and industry comment during consultation). *p < 0.05, bold: outside HCD range*

Dose in mg/kg bw/d **Tumour type (species/sex)**	**0**	**0.3**	**1.0**	**3.0**	**HCD range**
Malignant mesothelioma (rat/male) Overall rate (%) First incidence in days	 0/50 (0) -	 2/50 (4) 529	 2/50 (4) 728	 **5/50** **(10)*** 591	 0-8%
Hepatoblastoma (mouse/female) Overall rate (%)	 0/50 (0)	 **4/50 (8)**	 0/50 (0)	 **3/50 (6)**	 0-2% (2/250)
Hepatocholangiosarcoma (mouse/female) Overall rate (%)	 0/50 (0)	 0/50 (0)	 **1/50 (2)**	 **2/50 (4)**	 0/250
Hepatocellular carcinoma (mouse/female) Overall rate (%) First incidence in days	 12/50 (24) 638	 13/50 (26) 513	 10/50 (20) 440	 19/50 (38)* 599	 6-46%
Stromal polyp (mouse/female) Overall rate (%) First incidence in days	 0/50 (0) -	 1/50 (2) 729	 2/50 (4) 729	 **5/50** **(10)*** 409	 0-6%
Stromal sarcoma (mouse/female) Overall rate (%)	 0/50 (0)**°**	 0/50 (0)	 0/50 (0)	 **1/50 (2)**	 0/250

°industry noted that a single stromal sarcoma of unknown origin was also observed in the control group but was not considered by the study authors.

Increase in the incidence of malignant mesothelioma in male rats was not clearly dose-related and reached statistical significance only in the highest dose group, where it was also outside the historical control range but exceeded the historical control incidence only by one animal. Industry in their comment during consultation noted that *tunica vaginalis* mesothelioma is a neoplasm specific to F344 rats associated with a high background of Leydig cell tumours, which are rare in humans. However, as pointed out by the DS, the incidence of interstitial cell adenoma was not increased in the treatment groups as compared to controls (54, 34, 54, and 56% in controls, low, mid, and high dose, respectively). The study authors considered this finding an equivocal evidence of a carcinogenic activity of TMPTA.

The incidence of hepatoblastoma in female mice was clearly outside the corresponding historical control range in the low and high dose groups but lacked a dose-response relationship and did not reach statistical significance. A statistically not significant increase in the incidence of very rare hepatocholangiosarcoma was also observed in female mice outside the historical control range in the mid and high dose groups. A high background incidence was reported for hepatocellular carcinoma in these mice and although the increase in incidence reached statistical significance in the high dose group, it was inside the historical control range. A dose-dependent increase in the incidence of benign stromal polyps of the uterus was found in mice that was statistically significant and outside the historical control range in the high dose group. In this group, also one stromal sarcoma was observed, a tumour type not seen in historical and concurrent controls. Study authors deemed a stromal sarcoma of

unknown origin observed in the control group not treatment-related and concluded that there was some evidence of carcinogenic activity in female mice.

There are no mechanistic studies available investigating possible modes of action of TMPTA for the observed tumour types. The DS and industry in their public comment presented some hints that standard MoAs like CAR/PXR pathway, endocrine mechanism, or oxidative stress are not induced by TMPTA, but RAC considers these deliberations rather speculative.

RAC conclusion on classification

Since there are no human data available, Cat. 1A does not apply. If there are some indications of carcinogenic activity from animal studies, but evidence is not robust enough for Cat. 1B, then the guidance on classification requires a weight of evidence approach.

- Tumour type and background incidence: rare tumour types with low background incidences were observed in female mice (hepatocholangiosarcoma, stromal sarcoma) in very low incidences. Mesothelioma in male F344 rats are relatively common but were not accompanied by an increase in interstitial cell adenoma as was proposed for this strain.
- Multi-site responses: two sites were affected in female mice in the 2-year carcinogenicity study.
- Progression of lesions to malignancy: inconclusive - some observed tumour types are already malignant, benign precursors were not observed.
- Reduced tumour latency: No.
- Whether responses are in single or both sexes: tumours were reported in male rats and female mice.
- Whether responses are in a single species or several species: rats and mice were affected.
- Structural similarity to a substance(s) for which there is good evidence of carcinogenicity: none.
- Routes of exposure: dermal, relevant for human exposure.
- Comparison of absorption, distribution, metabolism and excretion between test animals and humans: inconclusive due to a lack of data on absorption of low doses in human skin.
- The possibility of a confounding effect of excessive toxicity at test doses: TMPTA exhibited a low systemic toxicity in carcinogenicity studies, but led to local inflammation of the site of application. Tumours accompanied by inflammation und hyperplasia occurred only in transgenic mice predisposed to skin lesions.
- Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity: no mechanistic studies available to exclude human relevance. Weak hints of a clastogenic effect *in vitro* were found in mutagenicity studies. No genotoxic effect was shown *in vivo*.

Although incidences reported were low, the tumour types observed in a guideline compliant carcinogenicity study are rare and, given a lack of mechanistic data, their relevance for humans cannot be excluded. Therefore, RAC concurs with the DS that **classification as Carc. 2; H351 is warranted.**

10.8 Reproductive toxicity

Not assessed in this dossier.

10.9 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.10 Specific target organ toxicity-repeated exposure

Not assessed in this dossier. Following data by dermal route of exposure are presented in regards to the classification proposal for carcinogenicity.

Table 18. Studies on repeated dose toxicity after dermal administration

Method	Results	Remarks	Reference
F344/N rats; 5/sex/dose 12.5 - 25 - 50 - 100 - 200 mg/kg bw (nominal per unit body weight) Vehicle: acetone 5 days per week for 16 days NTP protocol.	NOAEL local rats (males and females) < 12.5 mg/kg bw/day (epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, chronic active inflammation of the dermis). NOAEL systemic rats ≥ 200 mg/kg bw/day.	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)
B6C3F1 mice); 5/sex/dose 12.5 - 25 - 50 - 100 - 200 mg/kg bw (nominal per unit body weight) Vehicle: acetone 5 days per week for 16 days NTP protocol.	NOAEL local mice (males and females) < 12.5 mg/kg bw/day (epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, chronic active inflammation of the dermis). NOAEL systemic mice ≥ 200 mg/kg bw/day.	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)
Fischer 344 rats; 10/sex/dose	NOAEL systemic ≥ 12	2 (reliable with	NTP (2005)

0.75 - 1.5 – 3 - 6 - 12 mg/kg bw/day (nominal per unit body weight) Vehicle: acetone 5 days per week for 14 weeks Additional groups of 10 male and 10 female rats designated for clinical pathology testing received the same doses for 23 days NTP protocol	mg/kg bw/day NOAEL local male rats < 0.75 mg/kg bw/day (increase of epidermis hyperplasia) NOAEL local female rats = 0.75 mg/kg bw/day (hyperplasia of sebaceous gland)	restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	
B6C3F1 mice; 10/sex/dose 0.75 - 1.5 – 3 - 6 - 12 mg/kg bw/day (nominal per unit body weight) Vehicle: acetone 5 days per week for 14 weeks NTP protocol	NOAEL systemic ≥ 12 mg/kg bw/day NOAEL local male mice = 1.5 mg/kg bw/day (hyperplasia and degeneration of epidermis, chronic active inflammation of the dermis, hyperkeratosis and hyperplasia of the sebaceous gland) NOAEL local female mice = 0.75 mg/kg bw/day (chronic active inflammation of the dermis)	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)
rabbit (New Zealand White); 5/sex/dose 500 mg/kg bw/day (nominal per unit body weight) Vehicle: unchanged Exposure: Two weeks (Daily, five days/week) Six animals per group were sacrificed after 15 days and remaining 4 animals after 30 days. Animals were monitored for clinical signs and mortality, body weight gains, dermal reactions, gross pathology and histopathology.	NOAEL local < 500 mg/kg bw/day. It is not possible to adequately set a NOAEL for systemic effects since there is not enough information on incidence and severity of the clinical signs (decreased motor activity and nasal discharge) and decreased body weight.	3 (not reliable) experimental result **Test material (EC name): TMPTA** Purity not stated	Anonymous (1979)

Dermal studies:

In a NTP range finding study (NTP, 2005), F344/N rats or B6C3F1 mice were administered 0, 12.5, 25, 50, 100, or 200 mg TMPTA/kg body weight/day, 5 days per week for 16 days. All rats and mice survived to the end of the study. Mean body weights of dosed rats were similar to those of the vehicle controls. In mice, the body weight gain of high dose males was significantly reduced (without impact on body weight), while female body weight was significantly increased. Irritation at the site of application was most commonly seen in rats and mice administered 50 mg/kg bw/day or greater. Microscopically, non-neoplastic lesions occurred at the site of application in all dose groups. Animals showed statistically significant epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, chronic active inflammation of the dermis from the lowest dose, with severity (average severity grade of lesions in affected animals) increasing with doses. More severe lesions occurring generally at the higher doses and with severity also increasing with doses were ulceration, epidermal degeneration, and parakeratosis at the site of application.

Thymus weights of male mice administered 50 mg/kg bw/d or greater were significantly decreased. Histopathology detected thymic atrophy characterized by depletion of cortical lymphocytes in the two highest dose groups. Rats and female mice were not affected. Since thymus effects occurred in a context of severe dermal toxicity in male mice and were not consistently found in the NTP studies of longer duration, this effect seems rather due to stress than direct effect of the substance (Greaves, 2007). The systemic dermal NOAEL for rats and mice was ≥ 200 mg/kg bw/day. The dermal NOAEL for local effects in both species was < 12.5 mg/kg bw/day.

In the subsequent study, F344/N rats and mice were administered 0, 0.75, 1.5, 3, 6, or 12 mg TMPTA/kg body weight, 5 days per week for 14 weeks. No mortality and no difference in body weight were observed for mice and rats. Irritation at the site of application was noted at 12 mg/kg bw/day. Microscopically, epidermal hyperplasia occurred in all dosed groups of male rats. At higher doses (from 1.5 mg/kg bw/day), epidermal hyperplasia, degeneration, and necrosis (females only), chronic active inflammation of the dermis, hyperkeratosis, and sebaceous gland hyperplasia were reported in rats of both sexes at the site of application with a dose dependent increase in severity. Similarly, in mice, epidermal hyperplasia was observed at the site of application from 1.5 mg/kg bw/d in females and from 3 mg/kg bw/d in males. From 3 mg/kg bw/d in male mice and 6 mg/kg bw/d in female mice, increased incidences of the following non-neoplastic lesions also occurred at the site of application: hyperkeratosis, epidermal degeneration, chronic active inflammation of the dermis, and sebaceous gland hyperplasia. Epidermal suppurative inflammation, necrosis and dermal fibrosis occurred in male and female mice of the 12 mg/kg bw/d group. Haematology results indicated that TMPTA induced a neutrophil count increase at 12 mg/kg bw /d in both species that would be consistent with an inflammatory response related to the dermatitis observed histopathologically. Decreased lymphocytes counts in male rats at week 14 would be consistent with a stress-related response. Absolute and relative thymus weights of 12 mg/kg male rats, absolute thymus weights from 0.75 mg/kg bw/d in female rats and relative thymus weights at 0.75 and 12 mg/kg bw/d female rats were significantly decreased. As already mentioned above, this effect seems rather due to stress than direct effect of the substance. No effects on reproductive organs, sperm parameters (sperm count and motility) and estrous cycle were observed, except a significant decrease in left testis weight in rats at 12 mg/kg. Although the relative length of time spent in the oestrous stages differed significantly from vehicle groups at 6 and 12 mg/kg bw/d in female mice, the differences were not considered biologically significant. The systemic NOAEL after dermal exposure for 90 days in rats and mice is ≥ 12 mg/kg bw/day based on the lack of treatment-related effect. The NOAEL for local effects is lower than 0.75 mg/kg bw/d in male rats, equal to 0.75 mg/kg bw/d in female rats and mice and equal to 1.5 mg/kg bw/d in male mice (NTP, 2005).

The same findings were reported in a repeated dermal toxicity study of low reliability (Anonymous, 1979). New Zealand White rabbits received topical application of 0 or 500 mg/kg bw/d of TMPTA to the back, once daily for 5 days per week during 2 weeks. Six animals per group were sacrificed after 15 days and the remaining 4 animals after 30 days. Evaluation of treated skin revealed severe necrosis of the epithelium and upper dermis after 15 days and epithelial and sub epithelial dermal necrosis after 30 days. Motor activity was decreased and nasal discharge occurred in several animals in the treated group. Few animals exhibited slight body weight losses. Microscopic examination of selected tissues revealed no evidence of systemic toxicity resulting from administration of TMPTA.

10.11 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 19: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test) EU Method C.4-C (Determination of the "Ready" Biodegradability - Carbon Dioxide Evolution Test)	readily biodegradable % Degradation of test substance: 82 — 90 after 28 d (CO2 evolution) (range of 2 replicates)	1 (reliable without restriction) key study experimental result Test material (EC name): 2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate	Anonymous (2010)
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test)	readily biodegradable, but failing 10-day window % Degradation of test substance: 70 — 80 after 28 d (CO2 evolution)	3 (not reliable) supporting study experimental result Test material (EC name): 2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate	Anonymous (2004)

11.1.1 Ready biodegradability

The biodegradation of 20 mg/L of TMPTA by microorganisms from the activated sludge of a municipal sewage treatment plant was investigated according to OECD test guideline 301B under aerobic static exposure conditions (Anonymous, 2010). The biodegradability - based on CO_2 evolution - of the test substance was calculated to be 86% of the theoretical value ($ThCO_2$) after an incubation time of 28 days and reached 66% at the end of the 10-d window. Significant biodegradation of the test substance was observed after a lag phase of about 7 days. The positive control, sodium benzoate, reached 100% biodegradation after 14 days, thus confirming suitability of inoculum and test conditions. The test substance reached the pass level of 60% for ready biodegradability in the CO_2 Evolution Test (OECD 301B) within the 10-d window and, therefore, TMPTA can be termed as readily biodegradable.

The second available study for biodegradability showed that after 28 d, biodegradation values of 70-80% CO2/ThOD were reached classifing TMPTA as biodegradable (but failing the 10d-window) under aerobic environmental conditions. However, important information concerning the study could not be verified due to the lack of completeness of iuclid study summary, then this study is used as supportive data to support the ready biodegradability of TMPTA.

11.1.2 Hydrolysis

The abiotic degradation in water was tested with a close homologue of the registered substance, the ethoxylated TMPTA (Photomer 4149F, CAS 28961-46-5) according to OECD test guideline 111 (Anonymous, 2010). The Photomer 4149F was hydrolytically stable at pH4, slightly hydrolytically instable at pH 7, 20°C and 30 °C (DT_{50}=352 days and DT_{50}=113 days respectively). The Photomer 4149F is hydrolytically instable at pH 7, 50°C (DT_{50}=9.72 days) and pH 9 (DT_{50} = 4.54, 1.20 and 0.17 days at 20°C, 30°C and 50°C respectively) (Anonymous 2010).

11.1.3 Summary of data/information on environmental transformation

Regarding abiotic degradation, a close homologue of the registered substance the Photomer 4149F was hydrolytically stable at pH4, slightly hydrolytically instable at pH 7, 20°C and 30 °C (DT_{50}=352 days and DT_{50}=113 days respectively). The Photomer 4149F is hydrolytically instable at pH 7, 50°C (DT_{50}=9.72 days) and pH 9 (4.54, 1.20 and 0.17 days at 20°C, 30°C and 50°C respectively). In water, the test substance was readily biodegradable.

TMPTA is rapidly degraded in environmental conditions.

11.2 Environmental fate and other relevant information

Based on the log Kow of 4.35, the adsorption coefficient log Koc of the test substance was estimated to be 3.2 (Koc = 1585 L/Kg, KOCWIN v2.00). The calculated Henry's law constant of 0.06 Pa m^3/mol indicates very low volatility from surface waters.

11.3 Bioaccumulation

Table 20: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
QSAR estimation - OASIS CATALOGIC BCF base-line model v5.13.1	Log BCF = 0.63 BCF = 4.26 L/Kg	Updated CATALOGIC model with substances which incorporate acrylate fragments.	Anonymous (2018)

In the REACH Registration dossier, a BCF value of 344 L/kg was derived using the BCFBAF v.3.01 EpiSuite model. However, this estimation did not consider the surface active properties of TMPTA and the octanol-water partition coefficient Kow value used for this BCF estimation was unrelevant since surfactant properties of TMPTA. Consequently, a CMC-refined log Kow value calculated as the ratio between the solubility in octanol and the critical micelle concentration was provided (Anonymous, 2014). The relevant CMC-refined log Kow value for TMPTA is 4.35.

A new BCF value was estimated based on the CMC-refined Log Kow of 4.35. A weight of evidence approach has been proposed considering a BCF QSAR model battery approach and a QSAR Toolbox category approach (Anonymous, 2018).

First, a battery of available BCF QSAR models was used, i.e., BCFBAF (v3.01), T.E.S.T (v 4.2.1), VEGA (v 1.1.4) and OASIS Catalogic (v5.13.1) on several related or similar substances (the butyl acrylate and ethyl acrylate fragments of TMPTA, TMPTMA and its two fragments butyl methacrylate and ethyl methacrylate, and the Propylidynetrimethanol). The arithmetic mean and 75th percentile value of the BCF QSAR model battery is then calculated and used in the WoE approach.

Secondly, the QSAR Toolbox category approach was used to define a category of substances for TMPTA to derive a BCF based on available experimental data from substances of this category (i.e. 2-ethylhexyl fumarate, ethyl acrylate and pentaerythritol tetra(2-ethyl-hexanoate) with experimental log BCF of 2.39, 1.49 and 2.64 respectively). The integrated prediction methods within OECD QSAR Toolbox v4.2, i.e. data gap filling method 'nearest neighbour' and 'linear approximation' were used to calculate a BCF based on available experimental data.

Finally, the twofold derived BCF values for TMPTA were compared, and a final BCF was determined by applying the principle of weight of evidence.

Results showed that the 75th percentile of the log BCF values derived on the basis of the QSAR model battery are: 2 (TMPTA), 1.24 and 0.61 (butyl acrylate and ethyl acrylate fragments of TMPTA), 1.59 (TMPTMA), 1.47 and 0.81 (butyl methacrylate and ethyl methacrylate fragments of TMPTMA) and 0.47 (Propylidynetrimethanol). However, all the tested substances do not fit in most of the applicability domains of model battery, except for OASIS Catalogic (v5.13.1). Then, the 75th percentile of the log BCF values were not considered relevant.

Note that the OASIS Catalogic version used has been updated with an expanding training set including acrylate substances. All the tested substances fall into the applicability domains of this model, and the following Log BCF values were estimated: 0.61 and 0.59 (butyl acrylate and ethyl acrylate fragments of TMPTA), 0.70 (TMPTMA), 0.67 and 0.57 (butyl methacrylate and ethyl methacrylate fragments of TMPTMA) and 0.47 (Propylidynetrimethanol). For TMPTA, the QSAR Prediction Reporting Format generated by OASIS Catalogic v5.13.1 showed that the substance falls within the parametric domain of the model (log Kow, molecular weight, water solubility), as well as within its structural domain (85.71% of the fragments are recognised as correct). The prediction is inside the applicability domain of the model, and the estimated log BCF of TMPTA is 0.63.

The QSAR category approach made it able to estimate two log BCF values for TMPTA, i.e. 1.76 and 2.09 (calculation of nearest neighbour and linear approximation method respectively). However, this category has been built with only three substances among which two are not acrylate. Then this approach has not been considered sufficiently robust for BCF prediction.

In conclusion, the QSAR estimation with OASIS Catalogic model v5.13.1 was considered relevant and the estimated BCF of TMPTA is 4.26 L/Kg. All the tested substances show low estimated BCF values using OASIS Catalogic v5.13.1. Bioaccumulation properties assessed for other acrylates like MMA (RAR, 2002), HEMA (SIAR, 2001), HPMA (SIAR, 2006) concluded on the absence of bioaccumulation for these substances.

Then, TMPTA is considered as not bioaccumulative.

11.4 Acute aquatic hazard

Table 21: Summary of relevant information on acute aquatic toxicity

Method, Guideline, GLP status, Reliability	Species	Test material	Results[1]	Remarks	Reference
OECD 203 GLP RI 1 (reliable)	*Danio rerio*	Trimethylolpropantriacrylate	**LC_{50} (96 h): 0.87 mg/L** test mat. (meas; geom. mean) based on: mortality	-	Anonymous (2016)

EU Method C.1 (Acute Toxicity for Fish) (DIN 38412/15) Not GLP 3	*Leuciscus idus*	Trimethylolpropantriacrylate	LC_{50} (96 h): 1.47 mg/L test mat. (nominal) based on: mortality	-	(Anonymous, 1988)
EU Method C.2 (Acute Toxicity for Daphnia) RI 2	*Daphnia magna*	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate	LC_{50} (48 h): 19.9 mg/L test mat. (nominal) based on: mortality		Anonymous (1991)
EPA OPP 72-2 (Aquatic Invertebrate Acute Toxicity Test) RI 3	*Daphnia magna*	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate	LC_{50} (48 h): 19 mg/L test mat. (nominal) based on: mobility		Anonymous (1988)
EU Method C.3 (Algal Inhibition test) RI 2	*Scenedesmus subspicatus* (new name: *Desmodesmus subspicatus)*	Trimethylol propane triacrylate	ErC_{50} (96 h): 14.5 mg/L test mat. (nominal) ErC_{10}: 2.18 mg/L test mat. (nominal) based on: growth rate		Anonymous (1989b)

[1] Indicate if the results are based on the measured or on the nominal concentration

11.4.1 Acute (short-term) toxicity to fish

Two acute toxicity study of TMPTA on fish are available. The first one was performed by the REACH Registrant as part of a REACH Substance Evaluation decision (2016)[1]. In this study, TMPTA toxicity on *Danio rerio* under semi-static conditions for 96h was assessed according to the OECD 203 Guideline. The test organisms were *Danio rerio* (Teleostei, Cyprinidae), obtained on the 28 July 2016 from a recognised supplier: La Grande Rivière France (69490 Saint-Forgeux). All fish were in good health and free from any apparent malformation. Mortality of the batch was less than 5% in the week preceding the start of the study. No disease treatments were administered throughout holding and testing. Holding was maintained within the laboratory at a temperature of 21-25°C in glass tanks. A light cycle of 12 h light and 12 h dark was applied, illumination being provided by fluorescent tubes (intensity between 400 and 800 lux at the surface of the tanks). Tanks were aerated to ensure that the dissolved oxygen concentration is at least 60% of air saturation value in holding tanks and in test tanks. During holding, fish were fed twice per day with ground flake food TetraMin®. The fish were not fed for a period of 24h prior to test commencement or throughout the duration of the test. Fish were added to the test tanks within 30 min after the completion of preparation of the test solutions. During the test, pH varied between 8.0 and 8.1 and mean measured temperature: 21.1°C, min.: 20.0°C, max.: 21.8°C. The dissolved oxygen varied between 99.2-100% saturation. All test fish were weighed prior to the test and a representative number of test fish batch (10 at random) was measured after the test to assess compliance with guideline criteria. Fish were exposed to a series of test solutions renewed every day throughout the test period. Chemical analysis of the test item throughout the test period have shown instability of the substance. Therefore, the exposure concentrations were based on the geometric mean of measured concentrations 0.19, 0.41, 0.89, 1.71 and 3.10 mg/L. The 96h LC_{50} of TMPTA for the *Danio rerio* is 0.87 mg/L (measured concentration). At the end of the test, no mortality in the control was observed, the dissolved oxygen in the test tanks remained above 60% of the air saturation value and the pH did not vary by more than 1 unit. This study followed the good laboratory practices and fulfilled all validity criteria.

[1] https://www.echa.europa.eu/documents/10162/c826151d-81bb-4339-a377-c23a53007c11

In the *Leuciscus idus* study, the results showed that no mortality occurred at the first four concentrations (0.1, 0.215, 0.464 and 1 mg/L) whereas 100% of fish died at the highest tested concentration of 2.15 mg/L. Then, the geometric mean between the highest concentration without effect and the lowest concentration with 100% effect was proposed to determine an approximation of the LC_{50} of 1.47 mg/L (as stated in the OECD 203). These results are not consistent with the results observed in the two range-finding studies mentioned in the study report (Anonymous, 1988) where LC_{50} between 0.3 and 1 mg/L were detected. Furthermore, no concentrations were measured in this static acute study and the toxic effect relates to the nominal concentration of TMPTA. Consequently, this acute study on fish is not considered reliable and is used only as supportive data.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Two acute toxicity studies on daphnia are available (Anonymous, 1991; Anonymous, 1988). *D. magna* were exposed for 48h in a static system. Based on these two studies, TMPTA is considered to be moderately toxic to daphnia with EC_{50} = 19.9 mg/L (nominal concentration). Some information from these studies could not be verified (GLP conditions, no analytical measures), consequently they are used only as supportive data to show that aquatic invertebrates are less sensitive than fish.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

The test substance was tested for aquatic toxicity against the algae *Scenedesmus subspicatus* according to the method DIN 38412/9 (Anonymous, 1989b). Results are used as supportive data since some information in these study reports could not be verified (GLP conditions, no analytical measures). After 96h exposure the aquatic toxicity was determined to be: ErC_{10} = 2.18 mg/l and ErC_{50}=14.5 mg/L (nominal concentration). TMPTA is considered to be moderately toxic to algae which are less sensitive than fish.

11.5 Long-term aquatic hazard

No additional chronic data, other than the above algae EC_{10} of 2.18 mg/L is available.

11.6 Comparison with the CLP criteria

11.6.1 Acute aquatic hazard

The lowest $L(E)C_{50}$ obtained in acute aquatic toxicity studies is 0.87 mg/L in the fish *Danio rerio*. This value is below the classification threshold value of 1 mg/L. Consequently, TMPTA fulfils the criteria for classification as a acute hazard category 1, H400 to the aquatic environment, M-factor: 1.

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Based on the ready biodegradation test OECD 301B, TMPTA is readily biodegradable. Under environmental conditions, the substance is rapidly degraded. TMPTA has a log Kow of 4.35 and an estimated BCF based on log Kow of 4.26.

Chronic aquatic toxicity information is available for only one trophic level, the lowest available is ErC_{10}: 2.18 mg/L obtained in algae study. Therefore, according to the table 4.1.0 (b) ii of the CLP Regulation, this value is higher than 1 mg/L. Therefore, based on the chronic toxicity data, no classification is needed.

Nevertheless, as chronic data are available for only one trophic level, the proposed classification should also be compared with the classification based on acute data for the other trophic levels according to figure 4.1.1 and table 4.1.0 (b) (iii) of the CLP Regulation. The lowest L(E)C_{50} obtained in acute aquatic toxicity studies is 0.87 mg/L, in the fish *Danio rerio*. The substance is rapidly degradable in the environment and has a logKow of 4.35. No experimental BCF value is available. Since the lowest L(E)C_{50} is lower than 1 mg/L et the log Kow is >4, TMPTA fulfils the criteria for classification as a chronic hazard category 1, H410 to the aquatic environment. Since the conclusion is based on Table 4.1.0 (b) (iii), therefore the M-factor is based on the acute toxicity between 0.1 and 1 mg/l. In this case, the same factor M applies for both acute and long-term hazard, and M-factor: 1.

The most stringent outcome should be retain for classification. Thus, TMPTA fulfils the criteria for classification as a chronic hazard category 1, H410 to the aquatic environment, M-factor: 1.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the lowest aquatic acute toxicity value in fish **of less than 1 mg/L, TMPTA needs to be classified as acute hazard category 1, H400. T**he EC_{50} lies in the range for application of an M-factor of 1 (i.e., 0.1 <EC_{50}≤1).

I**n absence of adequate data on chronic toxicity and regarding the lowest EC_{50} lower than 1 mg/L, TMPTA needs to be classified as chronic hazard category 1, H410 and a** M -factor of 1 is applied **according to the Regulation (EC) No 1272/2008.**

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

TMPTA is a surface-active substance and is currently not classified for environmental hazards.

TMPTA is considered by the DS to be rapidly degradable as reported in a reliable ready biodegradability study, in which biodegradation reached 86% of the theoretical value ($ThCO_2$) after 28 days and 66% at the end of the 10-day window (OECD TG 301B). TMPTA has an estimated Log K_{OW} of 4.35 (25ºC) calculated as the ratio between the test substance solubility in octanol (499.900 mg/L, RRD, March 2020) and the critical micelle concentration (22.1 mg/L, RRD, March 2020). The estimated BCF based on Log K_{OW} of 4.35 was 4.26 L/kg, thus TMPTA was considered to have limited potential for bioaccumulation by the DS.

Only short term aquatic toxicity tests were available including all three trophic levels. The lowest LC_{50} was obtained in the acute aquatic toxicity studies was an LC_{50} (96h) value of 0.87 mg/L for the fish *Danio rerio*. This value is with the range of 0.1 < L(E)C_{50} ⩽ 1 mg/L and, consequently, the DS concluded that TMPTA fulfils the criteria for classification as acute hazard Category 1; H400 to the aquatic environment with an M-factor of 1.

No chronic aquatic toxicity studies were provided by the DS. A chronic endpoint based on the growth of algae was derived from the 96h study on *Scenedesmus subspicatus* as E_rC_{10}=2.18 mg/L. The DS concluded on chronic hazard classification and chronic M-factor by applying the surrogate approach (Annex I, 4.1.2.3, Figure 4.1.1 of the CLP Regulation) and taking into consideration acute toxicity data on *Danio rerio* (96h LC_{50} value of 0.87 mg/L). Taking into account the estimated Log K_{OW} of 4.35, which is greater than the Log K_{OW} of 4 CLP criterion,

the DS proposed the classification of TMPTA as Aquatic Chronic 1; H410, with an M-factor of 1.

Comments received during consultation

Comments were received from four MSCAs and one comment was received from industry. Two MSCAs explicitly supported the DS on the classification proposal. One MSCA supported the DS on the classification but requested justification on the appropriateness of the Log K_{OW} calculation method in relation to the substance properties and also a clarification on the appropriateness of the modelled BCF. Another MSCA requested a clarification on the reported key aquatic acute fish toxicity endpoint. Industry supported the DS classification proposal and provided some clarification on bioaccumulation wording which were accepted by the DS. All clarifications provided by the DS and all the points raised by the MSCAs and the industry can be found in the RCOM document.

Assessment and comparison with the classification criteria

Degradation

As reported in a ready biodegradability test (OECD TG 301B) (Reliability Index, RI =1) the biodegradability as CO_2 evolution of the test substance was calculated to be 86% of the theoretical value ($ThCO_2$) after an incubation time of 28 days and reached 66% at the end of the 10-day window. A second study on biodegradability (OECD TG 301B) was available but was rated as not reliable (RI=3) by the DS due to lack of available information and was used by the DS as supportive study. The study showed that after 28d, biodegradation values reached 70-80% of the theoretical value ($ThCO_2$), but failed to reach biodegradation above 60% of the theoretical value ($ThCO_2$) at the 10-day window. RAC notes that this study as reported in the TMPTA RRD complies with GLP and meets all the validity criteria and thus is rated with a RI=1 by the registrant (TMPTA, RRD, March, 2020). On hydrolysis, results of a close homologue of the TMPTA, the ethoxylated TMPTA (Photomer 4149F, CAS 28961-46-5), it was shown to be hydrolytically stable at pH4, slightly hydrolytically instable at pH 7, 20°C and 30 °C, while hydrolysis was observed at pH 7, 50°C and pH 9, 20°C, 30°C and 50°C. This would suggest that TMPTA might also hydrolyse in similar extent in these conditions. RAC agrees with the DS's conclusion that TMPTA should be considered to be rapidly degradable for classification purposes, based on the two ready biodegradability test results presented in the CLH report.

Bioaccumulation

TMPTA has an estimated Log K_{OW} of 4.35 (25°C) calculated as the ratio between the test substance solubility in octanol (=499,900 mg/L) and the critical micelle concentration (CMC, =22.2 mg/L, Dreyer, 2014). RAC agrees with the DS on Log K_{OW} derivation conclusion and notes that since TMPTA is a non-ionic surfactant (head-group), the refined CMC-refined Log K_{OW} can be considered reliable for classification purposes. Thus, the comparison of measured solubilities in octanol and water and use of the CMC in water as a solubility limit, in order to avoid the artefact of unrealistically Low K_{OW} values, is considered acceptable. This is an established working approach for surfactants and is also proposed in the Guidance on Information Requirements and Chemical Safety Assessment Chapter, R.7a, R7.1.8.5, when no experimental Log K_{OW} is provided.

No experimental BCF was available. A BCF value was estimated based on the CMC-defined Log

K_{OW} of 4.35. BCF values were derived by employing two QSAR approaches and considering a weight of evidence. RAC concludes that the QSAR category approach was not robust enough for classification purposes (see the Background Document for details).

The estimated BCF based on the Log K_{OW} of 4.35 was 4.26 L/kg and, thus, the DS proposed TMPTA to be considered to have limited potential for bioaccumulation. RAC disagrees with the DS and concludes that, based on the Log K_{OW} of 4.35 which is above the Log K_{OW} value of 4 CLP criterion, TMPTA should be considered as a potentially bioaccumulative substance. This has no impact on the classification as proposed by the DS. RAC notes that if new, good quality experimental BCF values are provided in the future, the proposed classification may need to be revisited. RAC also acknowledges that some uncertainties on the Log K_{OW} derivation from the critical micelle concentration may still remain.

Aquatic toxicity

Only short-term tests were available for three trophic levels. All the aquatic toxicity data are summarised in the Table below.

Table: *Summary of the relevant information on aquatic toxicity (key values represented in bold)*

Method, Guideline, GLP status, Reliability (RI)	**Species**	**Endpoint**	**Reference**
OECD TG 203 GLP, RI: 1	*Danio rerio*	**LC_{50} (96 h): 0.87 mg/L** test mat. (meas; geom. mean) based on: mortality	Anonymous (2016)
EU Method C.1 (Acute Toxicity for Fish) (DIN 38412/15) Not GLP, RI: 3	*Leuciscus idus*	LC_{50} (96 h): 1.47 mg/L test mat. (nominal) based on: mortality	Anonymous (1988)
EU Method C.2 (Acute Toxicity for Daphnia) RI: 2	*Daphnia magna*	LC_{50} (48 h): 19.9 mg/L test mat. (nominal) based on: mortality	Anonymous (1991)
EPA OPP 72-2 (Aquatic Invertebrate Acute Toxicity Test) RI: 3	*Daphnia magna*	LC_{50} (48 h): 19 mg/L test mat. (nominal) based on: mobility	Anonymous (1988)
EU Method C.3 (Algal Inhibition test) RI: 2	Scenedesmus *subspicatus*	E_rC_{50} (96 h): 14.5 mg/L test mat. (nominal) E_rC_{10}: 2.18 mg/L test	Anonymous (1989b)

Two **acute** toxicity studies of TMPTA on fish were available. The lowest effect endpoint was an LC_{50} (96h) value of 0.87 mg/L (measured concentration) of TMPTA for *Danio rerio* (Anonymous, 2016). Two acute toxicity studies of TMPTA on the aquatic invertebrate *Daphnia magna* were available, with an EC_{50} value of 19.9 mg/L (nominal concentration) being reported (Anonymous (1991). Lastly, after 96h exposure the aquatic toxicity on algae (*Scenedesmus subspicatus*) was determined to be E_rC_{50}=14.5 mg/L (nominal concentration) (Anonymous, 1989b). The studies reporting nominal toxicity values were not considered reliable by the DS and were used only as supporting evidence of the higher sensitivity of fish.

No additional **chronic** data were available apart from the aquatic toxicity test on algae (*Scenedesmus subspicatus*) where an E_rC_{10} (96h) value of 2.18 mg/L (nominal concentration)

was determined (Anonymous, 1989b).

Conclusion on classification

Based on an LC_{50} (96h) value of 0.87 mg/L (*Danio rerio*), which is below the 96h LC_{50} criterion value of below 1 mg/L, RAC supports classification of TMPTA as **Aquatic Acute 1**; **H400**. RAC also supports an **M-factor of 1** for aquatic acute toxicity since the LC_{50} (96h) value falls within the range of $0.1 < L(E)C_{50} \leq 1$ mg/L.

For chronic classification, due to the absence of adequate chronic toxicity data assessment followed the steps descripted in Annex I, 4.1.2.3 and the Figure 4.1.1 (CLP Regulation). Based on these steps, since only chronic data from one trophic level (i.e. algae) were available, assessment was performed for both the criteria given in the Table 4.1.0 (b) (ii) and the Table 4.1.0 (b) (iii) since adequate acute toxicity data were available for other trophic levels and could be used as surrogate.

The most stringent outcome is provided by considering the use of the acute toxicity data in a surrogate approach. As previously discussed, TMPTA has a Log K_{OW} value of 4.35, which is greater than a Log K_{OW} of 4 as the CLP criterion (no experimentally determined BCF was available). Therefore, the chronic classification and M-factor are based on the LC_{50} (96h) = 0.87 mg/L (*Danio rerio*) which is below the 96h $LC_{50} \leq 1$ mg/L criterion which result in the classification of the TMPTA as Aquatic Chronic 1, H410 with an M-factor of 1. In conclusion RAC supports the DS on the classification of TMPTA as **Aquatic Chronic 1**; **H410 with an M-factor of 1**.

Supplemental information - In depth analyses by RAC

The DS derived BCF values by employing two QSAR approaches and considering a weight of evidence approach.

The first approach employed a QSAR model battery (BCFBAF (v3.01), T.E.S.T (v 4.2.1), VEGA (v 1.1.4) and OASIS Catalogic (v5.13.1)) on several related or similar substances, namely the butyl acrylate and ethyl acrylate fragments of TMPTA, TMPTMA and its two fragments butyl methacrylate and ethyl methacrylate, and the Propylidynetrimethanol. BCF values derived on the basis of the QSAR model battery were not considered reliable by the DS, as all the tested substances did not fit in most of the applicability domains for BCFBAF (v3.01), T.E.S.T (v 4.2.1) and VEGA (v 1.1.4) models. In the contrary, it was reported by the DS that for the OASIS Catalogic (v5.13.1) model all the tested substances fall into the applicability domain of the model and also the TMPTA falls within the parametric and structural domain of the model.

Additionally, the DS reported that the model was updated with an expanding training set of substances including acrylate substances. Therefore, the BCF value of 4.26 L/kg (Log BCF=0.63) of TMPTA derived from the OASIS Catalogic (v5.13.1) model was considered reliable by the DS. RAC agrees with the DS on the assessment of the QSAR model battery approach and acknowledge the results (Log BCF=0.63 for TMPTA). However, RAC notes that despite the fact that the bioconcentration of non-ionic surfactants is of hydrophobic character and thus BCF estimation based on Log KOW could be appropriate, the QSAR model uncertainties still exist for surface-active substances and derived values shouldn’t be used to override valid Log KOW values. Additionally, RAC also notes that significant variability was shown in BCF values determined for the same surfactant with different species, and also when

the same surfactant was tested on the same species (Tolls et al. 1994 and Treu et. al., 2015).

The second approach employed QSAR Toolbox category approach (Anonymous, 2018). DS reports that experimental Log BCF values were available from the substances included in the category. The experimental Log BCF values reported for 2-ethylhexyl fumarate, ethyl acrylate and pentaerythritol tetra(2-ethyl-hexanoate) were 2.39, 1.49 and 2.64 respectively. RAC notes that only the value of 2-ethylhexyl fumarate was confirmed while for the other two substances calculated Log BCF values are reported in their respective RRD dossiers (accessed, March 2020) which differ from the ones reported by the DS. Taking this uncertainty into consideration and also as stated by the DS the fact that the category was built with only three substances among which two were not acrylates, RAC considers that the QSAR category approach was not robust enough for classification purposes.

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