Committee for Risk Assessment

RAC

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

Adopted

17 September 2020

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CLH-O-0000006856-61-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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**

The proposal was submitted by **France** and received by RAC on **28 June 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **12 August 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 October 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

Co-Rapporteur, appointed by RAC: **Kostas Andreou**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

This RAC opinion on proposed harmonised classification and labelling was adopted on **17 September 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 H400		**Add** M=1 M=1	
RAC opinion	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 H400		**Add** M=1 M=1	
Resulting Annex VI entry if agreed by 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	Skin Irrit. 2 Eye Irrit. 2 Skin Sens. 1 Carc 2 Aquatic Acute 1 Aquatic Chronic 1	H315 H319 H317 H351 H400 H410	GHS07 GHS08 GHS09 Wng	H315 H319 H317 H351 H400		M=1 M=1	D

GROUNDS FOR ADOPTION OF THE OPINION

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.

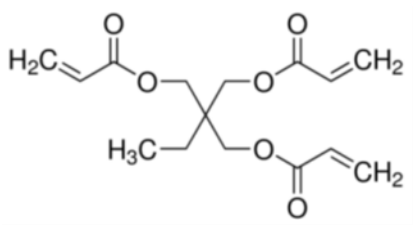

Figure: Chemical structure of TMPTA.

HUMAN HEALTH HAZARD EVALUATION

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 the 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.

<u>DS conclusion on classification</u>

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.

<u>Conclusion on classification</u>

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.

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.**

ENVIRONMENTAL HAZARD EVALUATION

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 (25°C) 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 $0.1 < L(E)C_{50} \leqslant 1$ 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^{o}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} ≤ 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**.

Additional references

Tolls J, Sijm DTHM, Kloepper-Sams P (1994) Surfactant bioconcentration - a critical review. Chemosphere 29:693-717

Treu G, Drost W, Jöhncke U, Rauert C, Schlechtriem C. (2015) The Dessau workshop on bioaccumulation: state of the art, challenges and regulatory implications. Environmental Sciences Europe. 27(1):34.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).