Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2-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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-ETHYL-2-[[(1-OXOALLYL)OXY]METHYL]-1,3-PROPANEDIYL DIACRYLATE; 2,2-BIS(ACRYLOYLOXYMETHYL)BUTYL ACRYLATE; TRIMETHYLOLPROPANE TRIACRYLATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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

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

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Substance 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
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		MemberState	1
Comment received				
In section 7, table 5 of the CLH report the following aspects have been noticed: • The given value for the log Kow is 4.35 at 25°C. According to the information on the EC-HA dissemination site (taken from the registration dossier) the log Kow is 4.35 at 20°C (since this calculated value is based on the substance solubility in octanol which was measured at 20°C). • For some physicochemical endpoints, the given values/results, references and comments do not correspond to the listed property. This applies to the following end-points: o Granulometry (given result: "Stable in organic solvents") o Stability in organic solvents and identity of relevant degradation products (given result: "The substance does not dissociate in water") o Dissociation constant (given result: "122 mPa.s (dynamic) at 20 °C") o Viscosity (given result: "Clear liquid") • In the confidential annex of the CLH report for all given substance compositions the EC No. given for the main constituent TMPTA (table 1 in all sections) is incorrect.				
Dossier Submitter's Response				
Informations on the Echa dissemination website are taken from registration dossier. These informations are not updated. Information on CLH report are the result of the evaluation of these informations. That is the reason why some informations from Echa website and CLH report are updates. Thank you for TMPTA EC number, CLH report will be updated.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	Belgium	ReachCentrum PARAD Consortium	Industry or trade association	2
Comment received				
ReachCentrum SA is submitting the comments concerning the CLH Proposal on behalf of the Polymerisable Acrylate Resins and Derivatives REACH Consortium and representing the registrants of 2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate; 2,2-bis(acryloyloxymethyl)butyl acrylate; trimethylolpropane triacrylate. ECHA note – An attachment was submitted with the comment above. Refer to public attachment TMPTA_PARAD_Comments_PublCons_October 2019.zip				
Dossier Submitter's Response				
FR: see response to comments 7, 12, 15				
RAC's response				
Noted				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	United Kingdom	Exponent International	Company-Manufacturer	3
Comment received				
Dermal tumours in the Tg.AC mouse are a measure of prolonged irritancy, not intrinsic carcinogenicity. This mouse model is no longer preferred by NTP, the sponsors of the study, due to a high false-positive rate. No dermal tumours were seen in F-344 rats or B6C3F1 mice (normal skin). Additionally, tumours of the forestomach are in a tissue not relevant to human. These tumour incidences, in an inappropriate model, are irrelevant to classification.				
Dossier Submitter's Response				
FR: Critical analysis of the results from the Tg.AC mouse model was already performed in the CLH report (table 10 page 20 and page 25/26). We recognize that this model cannot be used alone as the basis of classification proposal, due to its high sensitivity to dermal tumour promoter. However, some points should be highlighted: - Analysis of Tg.AC hemizygous mouse studies showed 77% accuracy in identifying known human carcinogens (Pritchard et al. 2003 cited in NTP 2012). - We agree that no dermal tumours were seen in standard carcinogenicity studies. At least 2 hypothesis can be made to explain this discrepancies: o 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. o skin tumours mainly occurred from 6 mg/kg bw/day in the Tg. AC mouse whereas the 2-year carcinogenicity study was performed at doses up to 3 mg/kg bw/day. - Regarding forestomach tumours in Tg. Ac mouse: it is noted in page 381 of the guidance on the application of the CLP criteria, that "*Tumours occurring in such tissues indicate that the substance has the potential to induce carcinogenic effects in the species tested. It cannot automatically be ruled out that the substance could*				

cause similar tumours of comparable cell/tissue origin in humans." In addition, it should be noted that these tumours do not result from a gavage exposure (but dermal) and cannot be due to prolonged high local concentrations.

In conclusion, 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. This is confirmed by the results of the standard carcinogenicity assays.

Anyway, the proposed classification is primarily based on the results of the 2-year carcinogenicity studies where uterine and liver tumours were reported for female mice and malignant mesothelioma from tunica around testis in male rats.

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	United Kingdom	Exponent International	Company-Manufacturer	4

Comment received

Rare liver tumours occur in a strain of mouse (B6C3F1) that gives a high profusion of liver tumours; at least 76% of control females had hepatocellular adenoma and/or carcinoma and at least 26% had multiple liver tumours. In treated groups, the incidence of hepatocellular adenoma and carcinoma were not statistically different to control. A comet assay in mouse liver showed no DNA damage. There is also no evidence of hepatotoxicity. In the TMPTA study, tumours of interest are restricted to females. Hepatoblastoma (HB) in females showed no dose relationship and all HB occurred in individuals that presented with another hepatocellular tumour. HB occurs occasionally in females of this strain but is more common in males. In male controls, the incidence of HB was higher than the mean of the historical control data (HCD), suggesting that the population of mice in this study is susceptible to spontaneous HB. Hepatocholangiocarcinoma (HCC) appears very rare in the female HCD, but in the HCD for males HCC appears to cluster: that is, in the few studies where HCC is reported in controls, multiple animals are affected in the study. The presence of 2 HCC in control males in the TMPTA study suggests the population of mice in this study is susceptible to spontaneous HCC. The high incidence of spontaneous liver tumours in this study makes this study unreliable for the assessment of hepatocarcinogenesis, as noted in the "Guidance on Application of the CLP Criteria". The apparent increases in HB and HCC in this study do not provide reliable evidence for classification.
This reasoning is more comprehensively explained in Exponent document 1602127.000-1225 (TMPTA_PARAD-Cons_Full text_Carcinogenicity_October 2019), submitted separately.

Dossier Submitter's Response

FR: Regarding incidence of liver tumours in the control female group, repartition of neoplasms in the liver of vehicle female group is as follow:

Summary of the Incidence of Neoplasms in Female Mice of Trimethylolpropane Triacrylate

	Vehicle Control
Alimentary System (continued)	
Liver	(50)
Carcinoma, metastatic, pancreas	
Fibrosarcoma, metastatic, skin	
Hemangiosarcoma	
Hepatoblastoma	
Hepatoblastoma, multiple	
Hepatocellular adenoma	11 (22%)
Hepatocellular adenoma, multiple	23 (46%)
Hepatocellular carcinoma	9 (18%)
Hepatocellular carcinoma, multiple	3 (6%)

The negative response in the lung from Comet assay suggest that neoplastic effects reported in female mice are not due to a genotoxic MoA (taking into account all limitations of this assay detailed in the CLH report).

You comment that "all HB occurred in individuals that presented with another hepatocellular tumour", however it is not the case for 2/4 animals at 0.3 mg/kg. In contrast to HB and HCC, hepatocellular adenoma/carcinoma are found at rather high incidence in mice. This is well-known and should not be used as an argument to dismiss the occurrence of rare tumours, such as hepatoblastoma and hepatocholangiocarcinoma.

Various effects were reported in the liver of female mice treated with TMPTA for 2 years: hepatoblastoma (0%, 8%, 0%, 6% - above HCD), hepatocholangiocarcinoma (0%, 0%, 2%, 4% - not seen in HCD) and hepatocellular carcinoma (positive trend: adjusted rate: 25.4%, 28.4%, 22.7%, 41.3%). Non-neoplastic effects, such as eosinophilic focus (statistically significant at 0.3 mg/kg) and Kupffer cell pigmentation (statistically significant at 1 mg/kg) were also observed.

We agree that the incidence of HB and HCC is rather high in vehicle males for these rare tumours, which is not the case for vehicle females. We cannot explain this sex difference but in the absence of robust explanation, it is not sufficient to disregard this study and corresponding results, in particular for rare tumours.

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	United Kingdom	Exponent International	Company-Manufacturer	5

Comment received

Uterine stromal polyp in female mice is a poor indicator of human carcinogenesis; the biology and etiology differ from that of humans (Davis, 2011). Statistical significance might be attributed to a control value below the HCD mean. There is an omission in the CLH report: the incidence of uterine malignant sarcoma is similar in control and high dose animals (a uterine sarcoma of uncertain origin – i.e, uncertain as to if it is of stromal

origin - was present in a control animal). Uterine stromal polyp in mice are insufficient basis for classification. Reference: Davis (2011): https://journals.sagepub.com/doi/pdf/10.1177/0192623311431466
Dossier Submitter's Response
FR: Discussion about the relevance of this finding – choice of the HCD, physiology between rodent and human - is presented on page 24 of the CLH report. The increases of "stromal polyp" and "stromal polyp or stromal sarcoma" (mainly driven by the increase of stromal polyps) are statistically significant. You mention that "statistical significance might be attributed to a control value below the HCD mean", however, even if it is the case, the incidence exceeds the HCD range at the highest dose. Only one uterine sarcoma is observed at the highest tested dose (0 in the control group), however, it should be noted that uterine sarcoma is a rare finding in dermal NTP studies (historical incidence: 0/250). We agree that the increase of uterine stromal polyp and the presence of one case of uterine sarcoma at the highest dose cannot be considered as a clear evidence of carcinogenic activity. Instead, NTP concluded as some evidence – we reach the same conclusion. Overall, The evidence of carcinogenicity of TMPTA is not sufficient to propose a classification Carc. 1B when considering each tumours separetely. Instead, the proposed classification as Carc. 2 is based on a weight of evidence considering all tumours reported in mice (liver and uterus) and rats (mesothelioma). The reference you cite in your comment is already present in the CLH report (page 24)
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	United States	Exponent International	Company-Manufacturer	6
Comment received				
Classification as Carc Cat 2 is not supported. The data for carcinogenicity are peculiar, require careful interpretation, and offer inadequate evidence for classification. Malignant mesothelioma of the tunica vaginalis (TVM) in the male Fisher-344 rat is highly site-specific and characteristic of this strain of rat. While mesothelium is a tissue lining the thoracic, abdominal and (in males) scrotal cavities, the F-344 shows a pronounced and peculiar susceptibility to mesothelioma specific to the tunica vaginalis. Maronpot et al. (2009, 2016) observes that in NTP F-344 studies where treatment-related TVM are reported, no mesothelioma occurred in females. It should be noted that the application site for TMPTA was between the shoulders; higher exposure might be anticipated in the thoracic, rather than scrotal, cavity. The lack of relevance of F344 rat TVM tumours to humans is discussed by Maronpot et al. (2009, 2016), who postulate an association between TVM and Leydig cell tumours (LCT) in the F-344 rat. The male F-344 rat has a notably high background incidence of LCT, as mentioned in the "Guidance on Application				

of the CLP Criteria". Although no dose-related change in the incidence of LCT was observed in the TMPTA study, 5 of 6 high dose TVM tumours occurred in individuals that were also identified as having LCT. This peculiarly site- and species-specific TVM in F-344 rats is concluded to be a spurious finding, irrelevant to classification.
The CLH report indicates that the association between TVM and LCT in this study should be dismissed because "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 knowledge gained in the two last decades, the evaluation has changed to-day and needs updating." The TMPTA Consortium is unaware of specific "new knowledge" in this context or how it affects the evaluation. The specific "new knowledge" and a detailed reasoning as to how it affects the evaluation should be specified in the CLH report. Further, Maronpot et al. followed up the 2009 publication with another in 2016 that reaffirms and updates the information presented in the 2009 paper.
References:
Maronpot et al. (2009) Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. Crit Rev Toxicol. 2009;39(6):512-537.
Maronpot et al. (2016):
https://www.tandfonline.com/doi/full/10.1080/10408444.2016.1174669

Dossier Submitter's Response

FR: Discussion on the relevance of malignant mesothelioma was already discussed in the CLH report (page 23).

Taking into account incidence of Leydig cells tumours (LCT) in the NTP study, no clear link can be deduced between tunica vaginalis mesothelioma (TVM) and LCT after TMPTA administration. Whereas TVM incidence increased with the dose, there is no increase of LCT. In your comment, you refer to CLP guidance: *the male F-344 rat has a notably high background incidence of LCT, as mentioned in the "Guidance on Application of the CLP Criteria".* If TVM are almost always associated to LCT, a high spontaneous incidence of TVM in male F-344 rats could be expected in this species. However, this is not the case. You also comment that "*5 of 6 high dose TVM tumours occurred in individuals that were also identified as having LCT*". In contrast, we can note that among the 7 animals with malignant mesothelioma across the treated groups (2 at 0.3 mg/kg bw, 2 at 1 mg/kg bw and 5 [not 6 as you mentioned] at 3 mg/kg bw), no LCT was observed in 3 of these animals.

In the CLH report, when we refer to "knowkedge gained in the two last decades", we only highlight that Maropont (2009) based its conclusion on old articles – that should be updated. Even if Maropont et al. published in 2016. This publication is still based his conclusion on NTP carcinogenicity studies performed up to 1998 with many references to Maronpot (2009).

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	Belgium	ReachCentrum PARAD Consortium	Industry or trade association	7

Comment received

The TMPTA Industry Consortium does not support classification as Carc Cat 2, and argues that the data for carcinogenicity are peculiar, require careful interpretation, and offer

inadequate evidence for classification.
Dermal tumours in the Tg.AC mouse are a measure of prolonged irritancy, not intrinsic carcinogenicity. This mouse model is no longer preferred by NTP, the sponsors of the study, due to a high false-positive rate. No dermal tumours were seen in F-344 rats or B6C3F1 mice (normal skin). Additionally, tumours of the forestomach are in a tissue not relevant to human.
Malignant mesothelioma of the tunica vaginalis (TVM) in the male Fisher-344 rat is highly site-specific and characteristic of this strain of rat. While mesothelium is a tissue lining the thoracic, abdominal and (in males) scrotal cavities, the F-344 shows a pronounced and peculiar susceptibility to mesothelioma specific to the tunica vaginalis. It should be noted that the application site for TMPTA was between the shoulders; higher exposure might be anticipated in the thoracic, rather than scrotal, cavity. The lack of relevance of F344 rat TVM tumours to humans is discussed by Maronpot et al. (2009, 2016), who hypothesis an association between TVM and Leydig cell tumours (LCT) in the F-344 rat. The male F-344 rat has a notably high background incidence of LCT, as mentioned in the "Guidance on Application of the CLP Criteria". Although no dose-related change in the incidence of LCT was observed in the TMPTA study, 5 of 6 high dose TVM tumours occurred in individuals that were also identified as having LCT. This peculiarly site- and species-specific TVM in F-344 rats is concluded to be a spurious finding, irrelevant to classification.
The CLH report indicates that the association between TVM and LCT in this study should be dismissed because "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 knowledge gained in the two last decades, the evaluation has changed to-day and needs updating." The TMPTA Consortium is unaware of specific "new knowledge" in this context or how it affects the evaluation. The specific "new knowledge" and a detailed reasoning as to how it affects the evaluation should be specified in the CLH report. Further, Maronpot et al. followed up the 2009 publication with another in 2016 that reaffirms and updates the information presented in the 2009 paper.
Rare liver tumours occur in a strain of mouse (B6C3F1) that gives a high profusion of liver tumours; at least 76% of control females had hepatocellular adenoma and/or carcinoma and at least 26% had multiple liver tumours. In treated groups, the incidence of hepatocellular adenoma and carcinoma were not statistically different to control. There is also no evidence of hepatotoxicity. In the TMPTA study, tumours of interest are restricted to females. Hepatoblastoma (HB) in females showed no dose relationship and all HB occurred in individuals that presented with another hepatocellular tumour. HB occurs occasionally in females of this strain but is more common in males. In male controls, the incidence of HB was higher than the mean of the historical control data (HCD), suggesting that the population of mice in this study is susceptible to spontaneous HB.
Hepatocholangiocarcinoma (HCC) appears very rare in the female HCD, but in the HCD for males HCC appears to cluster: that is, in the few studies where HCC is reported in controls, multiple animals are affected in the study. The presence of 2 HCC in control males in the TMPTA study suggests the population of mice in this study is susceptible to spontaneous HCC. The high incidence of spontaneous liver tumours in this study makes this study unreliable for the assessment of hepatocarcinogenesis, as noted in the "Guidance on Application of the CLP Criteria". The apparent increases in HB and HCC in this study do not provide reliable evidence for classification.
Uterine stromal polyp in female mice is a poor indicator of human carcinogenesis; the biology and etiology differ from that of humans (Davis, 2011). Statistical significance might be attributed to a control value below the HCD mean. There is an omission in the CLH report: the incidence of uterine malignant sarcoma is similar in control and high dose animals (a uterine sarcoma of uncertain origin – i.e, uncertain as to if it is of stromal

origin - was present in a control animal).
Overall, the tumour types observed in the TMPTA studies do not provide a reliable or adequate basis for classification. No classification should be applied.
Comments are addressing Sections 10.7 (p19), 10.7.1 (p20), 10.7.2 (p26) and 10.7.3 (p29) of the CLH Proposal

ECHA note – An attachment was submitted with the comment above. Refer to public attachment TMPTA_PARAD_Comments_PublCons_October 2019.zip

Dossier Submitter's Response

FR:

Comments on TMPTA toxicokinetics assessment: based on the *in vitro* study and according to EFSA guidance (EFSA, 2017), a dermal absorption of 0.8% was obtained for TMPTA. Only one high non-diluted concentration was used in this study. Therefore, it is unknown to what extent this value is relevant to lower concentrations. In particular, when considering the in vivo study in rats, it is clearly observed that dermal absorption increased with decreased concentrations. It is also noted, from the in vivo study in rats, that repeated dose exposure of TMPTA can enhance dermal absorption.This, maybe, partly, explain the large difference between this value from the in vitro study (0.8%) compared to the dermal absorptions obtained from the in vivo dermal studies in rats (up to 55%) and mice (75%).

Comments on genotoxicity: see responses to comments 10, 11, 12.

Comments on the Tg.AC study on TMPTA: see response to comment 3.

In addition, you note that "*the background incidence rate for skin squamous cell papilloma in Tg.AC mice at the NTP laboratories was reported to range from 0% to 40% in studies of 6 months duration*". In the case of TMPTA, the incidence of skin papilloma was more than 80% and up to 100% at 6 and 12 mg/kg bw/day in both males and females. Similarly for forestomach papilloma, you note that "*the historical control database from NTP shows spontaneous incidence rates for forestomach squamous cell papilloma of 0% to 47% in studies of 6 months duration*". In the case of TMPTA, the incidence of forestomac papilloma was more than 60% at 12 mg/kg bw/day in females. Therefore, in both cases, the incidences were statistically significant compared to control and clearly exceed the HCD.

You note that "*Likewise, the development of forestomach tumours also has been suggested to relate to increased irritation in this region of the digestive tract*." However, the route of administration of TMPTA in this study was by dermal route.

Comment on 2-year carcinogenicity study in rats and mice (NTP, 2012):
Table 6 in your comment: The severity of the hyperplasia of epidermis and of hyperkeratosis observed in rats was graded as minimal (1) by the NTP. Nonneoplastic lesions of the skin observed in mice were graded between 1 (minimal) and 4 (marked) depending on the effect. However, whereas the incidence of these findings increased with the dose, severity did not increase. See table 15 in Annex I of the CLH report.

Use of Acetone as solvent:

We agree that acetone is classified as EUH066. Therefore, it cannot be ruled out that the skin effects reported in the 2-year carcinogenicity study may be due, at least in part, to acetone. However, TMPTA is also classified as Skin irrit. 2 and thus can also be responsible to the skin effects reported in this study.

You comment that, in the OECD 422 study by oral route, no irritation in the forestomach is noted. First, it is not the same route of administration. Secondly, the vehicle used in the OECD 422 study is the PEG 400 for which we have some concerns about its capacity to mask the reactivity of TMPTA. The lack of local effects in this study is also in favour of this hypothesis since TMPTA is an irritating agent for which it could be not surprising to observe forestomac effect after oral administration.

You note that "*Acetone is additionally known to amplify the dermal absorption of some substances.*" You did not provide any reference to support this statement and did not specify what type substances you point in this sentence ("some substances"?).
As described above in our response, various other reasons can explain the differences between dermal absorption values from in vitro and in vivo studies.

Comment on the lack of systemic non-neoplastic toxicity in NTP dermal studies: We acknowledge that no systemic non-neoplastic effects were reported in carcinogenicity study in rats (NTP, 2012) and in studies in rats and mice for 5 days and 14 weeks (NTP, 2005). However, hyperplasia in the adrenal medulla (1/49, 4/49, 3/46, 10/50) was reported in male mice in the 2-year study (NTP, 2012). Increased incidence of eosinophilic focus and Kupffer cell pigmentation were observed, with statistical significance, in the 2-year study in female mice (without clear dose-response relationship)(NTP, 2012). Increased liver, kidney and heart weights, decreased lung weight, hematopoietic cell proliferation in various tissues (liver, spleen, mandibular, mediastinal, and mesenteric lymph node) and myelodysplasia were noted in the 6-month study in Tg.Ac mice.

Concerning the OECD 422 study you cite, it was not described in the CLH report since reprotoxicity is not part of the classification proposal. Please find below our analysis of this study as reported in our Substance Evaluation conclusion document (2019):
The second study is a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of TMPTA in PEG 400 in Crl:WI(Han) rats by oral route at dose levels of 0, 30, 100, 300 mg/kg bw/day (Unpublished study report 23, 2015). Males were exposed for 29 days beginning 2 weeks prior mating. Females were treated for 41-55 days (2 weeks prior mating until lactation day 4). This study followed the OECD guideline 422 set in 1996. However, it should be noted that this guideline was updated in 2016, in particular, to include endocrine parameters and to extend the duration of treatment until post-natal day 13 (which is thus not the case in the present study). Accuracy, homogeneity and stability of formulations were demonstrated. Only local irritating effect was reported. Irregular surface of the forestomach was noted in males at 100 mg/kg bw/day and in both sexes at 300 mg/kg bw/day with corresponding inflammation, squamous cell hyperplasia and/or ulceration. These findings were often accompanied by submucosal edema, new blood vessel formation and granulation tissue formation. In addition, hyper and/or parakeratosis was often present. […] TMPTA (in PEG 400) did not induce treatment-related effect in reproduction and development (Unpublished study report 23, 2015). There was a significant higher post-natal loss (loss of 5 pups in 3 litters on day 2) leading to a decrease in the viability index at 100 mg/kg bw/day (95.1% versus 100%). The increase in post-natal loss is not dose-related and remains within historical control data (2010-2015: P5-P95 = 0.00-1.00). It is noted that the solvents included in the historical control data were not clearly defined in the study report.

Comparison with these historical controls may be inappropriate considering the intrinsic properties of the PEG 400 used in the present study. Limitations of this study related to the choice of the solvent are detailed in the section dedicated to repeated-dose toxicity (5.6.3). In addition, it should be noted that an OECD 422 guideline is only a screening study which cannnot replace a full reproductive toxicity study such as an EOGRTS (OECD guideline 443) or a 2-generation study (OECD 416).”

Comment on malignant mesothelioma in male F-344/N rats:
See response to comment 6.

Tunica vaginalis mesothelioma are reported in humans (as a rare tumour), therefore, the relevance of this finding from rats to humans cannot be totally excluded, in the absence of adequate justification.

Regarding possible modes of action for the development of TVM, the following hypothesis were cited in the Maronpot publications: hormone imbalance and mechanical pressure as likely key events, but also a possible role of mitogenesis, oxidative stress and cycle alteration.

Genotoxic MoA for TVM: Table 9: the link between TVM and a possible genotoxic MoA cannot be investigated only based on results from an Ames test. From the 18 substances cited, 5 are negative in the Ames test according to your table (+ 1 if we consider TMPTA).

Non genotoxic MoA for TVM: see above our comments on the OECD 422 study. This is only a screening study (reduced powder to identify reproductive effects compared to a full reproductive study) with no hormonal measurement performed. Therefore, no conclusion can be made from this study regarding endocrine disturbance.

According to TOXCAST data, there are 15/20 active assay related to ER, 8/12 related to AR and 2/2 related to an inhibition of aromatase. Since TMPTA is active only at or above cytotoxicity limit, the results are considered as equivocal (rather than negative): https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID0027773#invitrodb-bioassays-toxcast-data

Regarding oxidative stress and chronic inflammation, this MoA was not adequately and specifically investigated to be ruled out.

Comment on hepatoblastoma and hepatocholangiocarcinoma
See response to comment 4

You state that " *Because of their [HB] co-occurrence with adenomas and carcinomas, they have been hypothesized to be derived from these tumour types*". However, this statement cannot be firmly confirmed and moreover, 2 HB were not associated with adenoma and carcinoma in the 0.3 mg/kg group.

It should be noted that Bach et al. (2010) is a summary of speacker’s presentations at the NTP satellite symposium in 2009. Regarding hepatocholangiocarcinoma, it refers to a presentation made by Rodney Miller from Experimental Pathology Laboratories. It is noted that "*Hepatocholangiocarcinomas were not considered to be treatment related in any study*". But it is not the case for TMPTA for which HB and HCC were considered related to treatment by the NTP (Bach publication was taken into account by the NTP in its conclusion on carcinogenicity of TMPTA (page 22 of the NTP report)).

You state that "*It [HB] is a tumour generally of late onset, consistent with the HB tumours observed in the TMPTA study, which were seen after 600 days in all affected animals and after 700 days in most*." From the NTP study, it can only be concluded that the first occurrence of HB is between 12 months (interim sacrifice) and 24 months for surviving animals.

Regarding non-neoplastic effects in the liver: Increased incidence of eosinophilic focus and Kupffer cell pigmentation were observed, with statistical significance, in the 2-year study in female mice (without clear dose-response relationship). In addition, in the NTP (2005) study with Tg. AC mice exposed for 6 months, were observed increased liver weight (statistically significant at 12 mg/kg in males for absolute weight and in females for absolute weight and relative weight) and hematopoietic cell proliferation in the liver (statistically significant at 12 mg/kg bw in both males and females). Therefore, your argument based on the absence of additional effect in the liver cannot be used to dismiss the occurrence of HB and HCC.

Regarding the hypothesized modes of action: there is no adequate and specific investigation to propose any kind of MoA for the occurrence of liver tumour induced by TMPTA. ToxCast data and results from the NTP studies are not sufficient to reach any conclusion. At least, it can be suggested that it is not mediated by a genotoxic mode of action based on the result of the Comet assay – with all limitations associated cited in the CLH report. Also, in ToxCast, some positive results are reported but no activation of CAR receptor. Thus the non relevance of the liver tumours has not been demonstrated.

<u>Comment on stromal polyp or stromal sarcoma of the uterus</u>
See response to comment 5

Since the NTP study with TMPTA used NTP2000 diet and since HCD is available with this diet, it is not appropriate to refer to HCD with NIH07 diet. In addition, for adequate interpretation, it is more relevant to use HCD contemporary to the NTP study with TMPTA.

Regarding to MoA: see above our comments on the OECD 422 study and on ToxCast results (endocrine activity). In addition, based on database available with TMPTA, no conclusion on the MoA for stromal polyps/sarcoma can be reached.

You state that "*Consistent with this, all of the stromal polyps observed in TMPTA-treated B6C3F1/N mice, with the exception of one that was seen in the high dose group at 409 days, developed after 658 days or more of treatment (NTP, 2005)*". From the NTP study, it can only be concluded that the first occurrence of stromal polyps is between 12 months (interim sacrifice) and 24 months for surviving animals.

<u>Further consideration</u>
The lack of skin tumours in the 2-year NTP studies is not a robust argument to totally exclude the various tumours reported in these studies.

You state on page 34: "*Finally, there is no known carcinogenic substance with a close structural relationship*." However, you not specify what are the "substances with a close structural relationship".

Overall, The evidence of carcinogenicity of TMPTA is not sufficient to propose a classification Carc. 1B when considering each tumours separetely. Instead, the proposed

classification as Carc. 2 is based on a weight of evidence considering all tumours reported in mice (liver and uterus) and rats (mesothelioma).
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		MemberState	8
Comment received				
Based on the available data the classification for Carc. 2 is supported. Two NTP studies on carcinogenicity were performed. One with rats and mice (2012) and one with transgenic mice (2005). The study from 2012 showed increased incidences of malignant mesothelioma (slightly above the historical control range) in male rats as well as increased incidences of hepatoblastoma (not dose-dependent), hepatocholangiocarcinoma and uterine stromal polyps or stromal sarcoma in female mice after dermal application. There were no neoplastic effects found in female rats and male mice. In this study there was no difference in survival reported but skin reactions such as hyperplasia, hyperkeratosis and chronic in-flammation were found in rats and mice. Transgenic mice showed increased incidences and multiplicity of squamous cell papilloma at the site of application in both sexes and forestom-ach squamous cell papilloma in female mice after dermal application (NTP, 2005). Overall, an increased incidence in malignant and benign tumours was found in female mice and an increased incidence in malignant tumours in male rats after dermal application. Fur-thermore, there is evidence of benign tumours in transgenic mice with one site in males and two sites in females. Although the mode of action is unknown and there was no common target organ identified in the two species and sexes, there is sufficient evidence of carcino-genicity of TMPTA for classification as Carc. 2.				
Dossier Submitter's Response				
FR: thank you for your support.				
RAC's response				
Noted				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		MemberState	9
Comment received				
Based on the available data the proposal for no classification is supported. Four bacterial reverse mutation assays (OECD TG 471) showed positive results for TA1535 in the presence of metabolic activation in two out of 4 assays but without a dose-dependency. Furthermore, four in vitro mammalian gene mutation assays (OECD TG 476) were reported either using L5178Y mouse lymphoma cells or CHO cells. TMPTA was found to induce gene mutations in mouse lymphoma cells without metabolic activation but not in CHO cells. In the same studies chromosomal aberrations were reported for mouse lymphoma cells and CHO cells as well as increased micronucleus frequencies in mouse lymphoma cells. However, the positive results in these assays were found in the presence of various degrees of cytotoxicity. Positive results were also obtained in an in vitro mammalian chromosome aberration test (OECD TG 473) using human lymphocytes although as above in the presence of cytotoxicity.				

Furthermore, in vivo assays were reported, namely in vivo micronucleus assay (OECD TG 474) and in vivo mouse alkaline Comet assay (OECD TG 489). The micronucleus assay showed negative results, but the result is questionable as there is no evidence if the target tissue was reached. For the in vivo Comet assay negative results were reported for the liver which is a target tissue of carcinogenicity but increased mean tail intensities were found in the bone marrow although not dose-dependent. However, the validity of the assay was ques-tioned in the dossier as a very short sampling time after treatment and an unusual solvent (PEG 400) which may be influencing the reactivity of TMTPA were used. Overall, there are some limitations to the data presented as positive results in vitro were found in the presence of cytotoxicity or without a dose-dependent effect and the validity of the in vivo assays is questionable. Therefore, the data presented are not sufficient for classi-fication.
Dossier Submitter's Response
FR: Thank you for your comment.
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
25.09.2019	Iceland		Individual	10
Comment received				
Comments attached on the Germ Cell Mutagenicity of trimethylolpropane triacrylate (CAS Number: 15625-89-5), pages 10-18 of the CLH report. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments on the CLH report for TMPTA final.docx				
Dossier Submitter's Response				
FR: - Comment on the "Anonymous 2006" study: you state that "*it is higly likely that the bone marrow was exposed*" with reference to the physicochemical properties of TMPTA. However, there is no experimental study with TMPTA allowing this statement (for example by a specific dosage of the molecule in the bone marrow either in the micronucleus assay or in any other study). In addition, this is not the only one limitation of this study. Indeed, the 5 analyzable animals/sex recommended by the OECD guideline were not reached for males in the vehicle group (group: 48 hour after treatment) and at 437.5 mg/kg bw (group: 24 hour after treatment) and for females at 2000 mg/kg bw (group: 48 hour after treatment) because of deaths due to dosing errors (one in each of these groups). This can decrease the statistical powder of the analysis. - Comment on the NTP (2005) study: as you mention, according to OECD guideline, a positive control substance "*may be waived when the laboratory has demonstrated proficiency in the conduct of the test and has established a historical positive control range*". However, in the NTP (2005) study, there is neither a positive control group nor historical positive control range provided. All these results and limitations had been discussed during a Member State Committee (MSC) meeting in April 2016, in which it was agreed that a further assay is needed to clarify the genotoxic concern of TMPTA. In the final decision from ECHA (06/07/2016), the test requested by the industrials was an "*In vivo Mammalian Alkaline Comet assay in*				

mice (test method: OECD 489) analysing bone marrow and liver, via parenteral route using injection techniques appropriate for irritating substances"

- Comment on the Comet assay:

    - PEG400:

We understand the technical problem related to the physicochemical properties of TMPTA and to the route of administration: i.e. find a solvent in which TMPTA was miscible and which can be injected by intravenous route. Please note that in the NTP (2005) study, TMPTA was injected using a mixture of absolute ethanol, Emulphor and phosphate-buffered saline. We agree that literature reported that PEG400 is well tolerated in experimental animals. Our main issue is not related to toxicity of PEG400 but rather to the anti-inflammatory / antioxidant properties of PEG 400 and also the possible chemical interactions between PEG 400 and TMPTA (see references in the CLH report). Indeed, PEG 400 may mask the reactive groups of TMPTA leading to a reduced toxicity of TMPTA but also can change its behaviour/kinetics in the organism.

Regarding the toxicity reported in the experiment 2 (experiment 1 is judged non valid since inadequate results for achieved concentration and homogenicity were noted): some clinical signs were reported among controls and treated groups, without a clear effect that can be linked to TMPTA administration: "*Clinical signs were generally limited to immediately following the first dose and included prostrate (1, 0, 1, 0 females at 0, 5, 10 and 20 mg/kg/day respectively), rapid and/or gasping respiration (0, 1, 4, 3 females respectively), staggering (0, 1, 0, 1 females respectively), lethargic (0, 0, 1, 2 females respectively) and dark eyes (0, 0, 1, 1 females respectively). Three animals (Animals M0505 and M0506, vehicle control and Animal M0706, 10 mg/kg/day) had a minor convulsion immediately after dosing on either Day 1 or 2, but recovered and were kept on the study*." In addition, there was no effect on body weight, on clinical chemistry and on histopathology, that can be related to treatment. Therefore, the doses tested cannot be considered as toxic.

    - Sampling time

See response to comment 11.

    - Interpretation of results

According to OECD guideline, a test chemical is considered clearly negative, providing that all acceptability criteria are fulfilled, if:
a) none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control,
b) there is no concentration-related increase when evaluated with an appropriate trend test
c) all results are inside the distribution of the historical negative control data for a given species, vehicle, route, tissue, and number of administrations
d) direct or indirect evidence supportive of exposure of, or toxicity to, the target tissue(s) has been demonstrated.

For TMPTA, criteria a) is not fulfilled since the group exposed to 5 mg/kg bw exhibits a statistically significant increase in the bone marrow compared with the concurrent negative control (experiment 2). Regarding criteria c), even if the increased mean tail intensity values reported in bone marrow remained within the historical control, comparison with these HCD is not considered relevant (only on 5 animals exposed orally to CMC (and not PEG 400 administered by IV route as in the present study) and tail

intensity mean in the bone marrow with PEG 400 lower than that reported with these HCD)(see CLH report page 20 for details). Therefore, the response is neither clearly negative nor clearly positive (i.e not all criteria are met).

According to OECD guideline, in case the response is neither clearly negative nor clearly positive and in order to assist in establishing the biological relevance of a results, further investigation could be performed such as scoring additional cells or performing a repeat experiment using optimised experimental conditions. Unfortunately, this was not performed by the laboratory in the case of TMPTA. This could have been particularly useful to confirm or not the biological relevance of the effect observed in the bone marrow at 5 mg/kg bw.

Therefore, based on the available Comet assay, it cannot be concluded that TMPTA is not genotoxic.

- Dose formulation analysis

We agree that experiment 1 cannot be considered valid due to inconsistencies in the dose formulation. Experiment 2 was taken into account in our evaluation. However, several biais (solvent used, sampling time, interpretation of the positive result obtained in the bone marrow) do not allow a firm conclusion on genotoxicity of TMPTA.

- Statistical analysis

The choice of the test used for statistical analysis is primordial for the interpretation of the results since a statistically significant increase of DNA damage is one of the criteria for concluding on the Comet assay. The absence of a dose-response relationship is not a sine qua non condition for concluding on the lack of genotoxicity but only one criteria (criteria b) described above) in the interpretation of the results.

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
07.10.2019	United Kingdom		Individual	11

Comment received

Attached comments refer to the germ cell mutagenicity section, pages 10-18 of the CLH report.

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

Dossier Submitter's Response

FR:

- Comment on in vitro genotoxicity studies: we note that you agree that TMPTA is a genotoxic agent in vitro.

- Comment on micronucleus assays:

"Anonymous 2006" study:

You note that "*Whilst there were reduced numbers of animals in several treatment groups (4 rather than 5), both male and female animals were used, hence there were twice the number of individuals at each dose level and as such no loss of statistical significance*." However, OECD guideline 474 state: "*Group sizes at study initiation should be established with the aim of providing a minimum of 5 analysable animals of one sex, or of each sex if both are used, per group*."

Concerning the toxicity found in this study: In males, no clinical signs and no mortality attributed to the treatment were observed in vehicle controls and low dose males. At 875 mg/kg bw, piloerection was noted and at 1750 mg/kg bw, two males were found dead after 24 h. Piloerection was noted in the surviving males. In females, no clinical signs or mortality were observed. Finally, deaths due to dosing errors were reported for 1 male in the vehicle control group, 1 male at 437.5 mg/kg and 4 females at 2000 mg/kg. Overall, in males, only piloerection is observed and the cause of the 2 deaths of males at 1750 mg/kg bw is not reported (it is thus not possible to link the mortalities to TMPTA administration). In females, no sign of toxicity is noted.

See also responses to comment 10.

- Comment on the Comet assay

PEG 400 as vehicle:
You comment the protocols used in the Ackland et al., Juarez-Moreno and Hodoshima publications. We note that the protocols used by these authors and the Comet assay are not similar: objectives are clearly not the same. Reference to these publications in the CLH report is only to point to possible anti-inflammatory and antioxidant / protective properties of PEG400 reported in the literature.

Regarding the two other publications you did not found:

- F. Bartoli Klugmann, G. Decorti, F. Mallardi, S. Klugmann, L. Baldini. Effect of polyethylene glycol 400 on Adriamycin toxicity in mice. Eur. J. Cancer Clin Oncol. Vol 20. No. 3. Pp. 405-410. 1981
- Bing-Liang Ma, Yan Yang, Yan Dai, Qiao Li, Ge Lin, Yue-Ming Ma. Polyethylene glycol 400 (PEG 400) affects the systemic exposure of oral drugs based on multiple mechanisms: taking berberine as an example. RSC Adv., 2017, 7, 2435-2442

All the publications cited on PEG 400 in the CLH report raise doubt about the adequacy of using this solvent in experimental toxicological assays. We agree that, at the time being, a definitive conclusion on the influence of PEG 400 on TMPTA toxicity cannot be reached, in the absence of specific investiguation. However, from literature search, it is suspected that using PEG 400 may mask/decrease the reactivity of TMPTA. In addition, we consider that PEG 400 is not a standard solvent for toxicological studies. First, it is not cited in OECD guideline. Secondly, some experts we contacted considered this solvent as not usual and even not adequate in toxicity studies. Finally, when we contacted ECHA in 2018, only one example of in vivo comet assay using PEG 400 as solvent was sorted from studies submitted under CCH/TPE. According to OECD guideline 489: "*Vehicle/negative control data should be collected so as to demonstrate reproducibility of negative data responses, and to ensure that the technical aspects of the assay were properly controlled or to suggest the need to re-establish historical control ranges*". Currently, it is not possible to demonstrate reproducibility of the negative response of PEG 400 based on the insufficient historical database available with this substance. In conclusion, we consider that more research is needed before using this solvent in toxicological studies.

OECD 489 adherence:
Sampling time: We agree that, based on the NTP (2005) data, sampling time at 30 minutes is more relevant to 2-6 hour after treatment, after IV administration. Some restrictions could be noted: different protocol (for example, bolus IV in the NTP versus slow infusion in the Comet assay) and species used (rat in the NTP study versus mice in the Comet assay). As noted in the CLH report, an adequate kinetics study in the Comet assay (avoiding impact of condition of administration, dose dose, vehicle) should have been particularly useful to confirm the relevance of the sampling time.

HCD: Considering the concerns raised by the use of PEG 400 (possible interference with TMPTA, solvent not commonly used in toxicology), it is more appropriate to consider historical control using this vehicle.

Interpretation of results: You note that *"This increase is small, well within historical ranges (despite this range being from animals treated via a different route and vehicle)."* We would like to highlight that the historical control data for vehicle (CMC) described in the study only consist on 5 animals and cannot be considered as robust data (see Annex I of the CLH report).

From Table 12.7 in your comment, we can note that control values range from 0.10 to 0.32. In comparison, in animals exposed to 5 mg/kg bw, there are 4/6 animals with tail intensity higher than 0.32 (0.48, 0.54, 0.82 and 1.71).

See also response to comment 10.

Conclusion: in contrast to your comment, the CLH did not reject all the genotoxicity studies. However, we note that all studies present deviations in protocols and/or in interpretation of results. From the data available, we cannot conclude that TMPTA show a lack of DNA and chromosome damage in vivo. Significant increase of DNA damage is noted in the bone marrow in the Comet assay. This effect is not dose-related, but in the absence of additional investigation, the biological relevance of this finding cannot be ruled out. Considering OECD criteria (guideline 489), the response is neither clearly negative nor clearly positive (see response to comment 10).

RAC's response

Noted

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	Belgium	ReachCentrum PARAD Consortium	Industry or trade association	12

Comment received

The TMPTA Registrants agree that no classification for mutagenicity is required for TMPTA. The mutagenic, clastogenic, and aneugenic properties of TMPTA were adequately investigated both in vitro and in vivo. In vitro, TMPTA primarily induced clastogenicity, but such an effect was not evident in vivo in OECD test guideline compliant studies. The in vivo comet assay is believed to be a reliable indicator assay for detecting gene mutagens as well as clastogens and this assay was negative with TMPTA. Thus, no data gaps were identified, and the database is sufficient to comprehensively assess the genotoxicity of TMPTA. Based on the available data, it is concluded that although TMPTA is an in vitro clastogen at cytotoxic concentrations, no such activity is likely to occur under normal in vivo conditions because of the cellular protective mechanisms operating in an intact

animal. The Comet assay performed on TMPTA followed the OECD Test guideline n°489 (2016) is reliable and conclusive. The study design, required by ECHA, included intravenous treatment of female mice and analyses of liver and bone marrow cells. Based on these Comet results, it was concluded that TMPTA did not induce biologically relevant increases in tail intensity in the liver or bone marrow when treated up to 20 mg/kg/day in female mice, considered as the maximal tolerated dose. Comments are addressing Sections 10.6.1 (p15), 10.6.2 (p17) and 10.6.3 (p18) of the CLH Proposal ECHA note – An attachment was submitted with the comment above. Refer to public attachment TMPTA_PARAD_Comments_PublCons_October 2019.zip
Dossier Submitter's Response
FR: comments on Comet assay: see responses to comments 10 and 11.
RAC's response
Noted

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	Belgium		MemberState	13
Comment received				
The Belgian CA supports the proposed environmental classification of trimethylolpropane triacrylate with: Aquatic Acute 1, H400; M=1 Aquatic Chronic 1, H410; M=1 Degradation Based on the results of a readily biodegradation study, it can be decided that the substance is readily biodegradable (>60% CO2-evolution). Bioaccumulation We agree with the conclusion of the DS. Following the CLP-guidance, for surface-active substances a QSAR estimated value of Kow or an estimate based on individual n-octanol and water solubilities should be provided instead of an analytical determination of Kow. According to the decision scheme in the CLP-guidance, if no valid/high quality experimentally determined BCF value is available and no valid/high quality experimentally determined log Kow, the use of validated QSAR estimations of Log Kow should be used. The CMC-refined (ratio between solubility in octanol and critical micelle conc) log Kow for TMPTA is 4.35, no experimental BCF is available only BCF estimations. Therefore it could be decided on the basis of a Log Kow >4 that the bioaccumulation criterion is not met. Aquatic Toxicity • Acute The most sensitive species in acute toxicity studies is fish with a 96hLC50 of 0.87 mg/L which warrants a classification with Aquatic Acute 1, H400 and M-factor of 1. We question however the reliability of the Daphnia study (Anonymous, 1991) as values are reported as nominal while no analytical monitoring was performed.				

• Chronic Based on the most stringent outcome of the NOEC (algae) and the surrogate approach (no chronic toxicity data for fish and invertebrates + bioaccumulation potential) the substance should be classified as Aquatic Chronic 1, H410 with M-factor of 1.
Dossier Submitter's Response
FR: Thank you for your comment. As stated in the CLH report, since some information from the invertebrate studies could not be verified, they are used only as supportive data to show that aquatic invertebrates are less sensitive than fish to TMPTA exposure ($LC_{50, fish}$ is 13 fold lower than $EC_{50, daphnia}$).
RAC's response
RAC agrees with the DS response and also notes the support.

Date	Country	Organisation	Type of Organisation	Comment number
09.10.2019	Germany		MemberState	14
Comment received				
We support the proposed classification as Aquatic Acute 1, H400 (M=1) and Aquatic Chronic 1, H410 (M=1). For classification purposes no QSAR estimated BCF values should be used. According to the Guidance on the Application of the CLP Criteria only experimentally determined BCF or log KOW values or QSAR estimated log KOW values should be considered.				
Dossier Submitter's Response				
FR: Thank you for your comment. Indeed and according to the Annex III.5 decision scheme from the Guidance on the Application of the CLP Criteria (2017), since no experimental BCF value and no experimental Log Kow value are available, the log Kow of 4.35 based on QSAR estimating has been considered.				
RAC's response				
RAC agrees with the DS response and also notes the support.				

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	Belgium	ReachCentrum PARAD Consortium	Industry or trade association	15
Comment received				
The Polymerisable Acrylate Resins and Derivatives (PARAD) REACH Consortium do not agree with the arguments in relation to bioaccumulation as presented in the CLH Proposal. The PARAD REACH Consortium, on different grounds, supports the proposed environmental CLH classification of acute hazard category 1, H400 and chronic hazard category 1, H410 (M-factor 1) according to Regulation (EC) No 1272/2008 (CLP) which is identical to registrants' environmental self-classification of TMPTA. Comments are addressing Sections 11.1 (p32) to 11.7 (p37) of the CLH Proposal ECHA note – An attachment was submitted with the comment above. Refer to public attachment TMPTA_PARAD_Comments_PublCons_October 2019.zip				
Dossier Submitter's Response				
FR: <u>Comment on criterion for environmental hazard classification</u>				

You support the proposed environmental CLH classification H400-H410 (M-factor 1), but you stressed that you *"overall do not agree to the approach of using log Kow as substitute for a relevant in-silico BCF for chronic hazard classification purposes…/… well-assessed non-experimental BCFs must become an accepted criterion for environmental hazard classification as applied in the PBT assessment according to Regulation (EC) No 1907/2006 (REACH)."*
The relevant available BCF for TMPTA was determined from QSAR estimation and was concluded to be 4.26 in our Substance Evaluation conclusion document (2019). However, the Log Kow of 4.35 has to be used as a criterion for the environmental chronic classification when no experimental BCF are available to be in accordance to the Regulation (EC) No 1272/2008 (CLP). Consequently, this log Kow value of 4.35 was used to propose the H400 and chronic hazard category 1, H410 (M-factor 1) for TMPTA.

Comment on the derived relevant BCF

Discussion on the concluded *in silico* BCF of 4.26 is presented in the CLH report (page 34-35). The WoE approach proposed a more conservative BCF value of 123 but was not considered relevant: the QSAR Toolbox category approach is based on a category built with only three substances among which two are not acrylate. Furthermore, regarding the model battery approach, the tested substances do not fit in most of the applicability domains of models, except for OASIS Catalogic (v5.13.1) which was updated with an expanding training set including acrylate substances (85.71% of the fragments recognised as correct). Then, the estimated BCF value of 4.26, based on the CMC-refined Log Kow of 4.35 (considering its surface active properties), and derived from a single model (OASIS Catalogic v5.13.1) has been considered as the relevant value for TMPTA (Substance Evaluation conclusion document (2019)).

CLH report Section 11.6.2 - Potential misleading

FR: we agree that in first paragraph, second sentence: "TMPTA has a log Kow of 4.35 and an estimated BCF based on log Kow of 4.26." The sentence could be corrected to "TMPTA has a log Kow of 4.35 and an estimated BCF of 4.26 based on log Kow of 4.35."

RAC's response

RAC agrees with the DS response and also notes the support.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2019	United Kingdom		MemberState	16

Comment received

2,2-bis(acryloyloxymethyl)butyl acrylate; (EC: 239-701-3; CAS: 15625-89-5)
We agree with the proposal but note the following points in relation to assessment of bioaccumulation.

The CLH report includes a CMC-refined log Kow value of 4.35 calculated as the ratio between the solubility in octanol and the critical micelle concentration. This estimated Kow may be more appropriate than an experimental log Kow value due to the surface active properties of the substance. The DS should provide justification for this calculation method which is only appropriate for specific types of surfactants depending on the

charge of the headgroup.

The CLP Guidance only refers to the use of experimental and not estimated BCF values. Without assessing the BCF QSARs presented in the CLH report in detail, it is unclear to us whether they are suitable to predict the bioaccumulation of this substance (the influence of the surfactant class and alkyl chain lengths is not discussed). Unless the RAC considers that the justification for this modelled BCF value is appropriate, it may be better to only use the modelled log Kow of 4.35 which meets the criteria for bioaccumulation potential under CLP.

Dossier Submitter's Response

FR: According to guidance on information requirements and chemical safety assessment (Chapter R7a Endpoint specific guidance, p 78-79), none of the experimental methods is very well suited for determining the Kow of surface active substances. Kow was previously measured according to shake flask method. As this shake flask method is the least suitable experimental method for surfactants and as indicated in the guideline, we have estimated that it would be more suitable to compare the measured solubilities in octanol and water. Considering surfactant properties of TMPTA, critical micelle concentration in water (CMC) was considered as a solubility limit. Kow value was finally estimated as the ratio between the test substance solubility in octanol and the CMC.

The BCF QSARs approach presented in the CLH report (page 34-35) was assessed during the evaluation process of TMPTA, in which the OASIS Catalogic v5.13.1 model updated with acrylates substances were applied. For the reasons presented above (see comment 15), predictions from model OASIS Catalogic v5.13.1 were considered the only relevant ones. Indeed, the substance falls within the parametric domain of this model (log Kow, molecular weight, water solubility), as well as within its structural domain (85.71% of the fragments are recognised as correct). The concluded estimated BCF of TMPTA should be 4.26 L/Kg (Substance Evaluation conclusion document (2019)). Note that 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.

RAC's response

RAC notes the support and agrees with the DS's response in regards to the BCF derivation part of the comment. However, RAC notes that DS didn't respond to the comment concerning the appropriateness of the Log K_{OW} calculation in respect to specific types of surfactants depending on the charge of the headgroup. RAC sees the Log K_{OW} as descriped in the CLH report as appropriate since the TMPTA is a non-ionic surfactant.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2019	Sweden		MemberState	17

Comment received

p. 35-36 Acute aquatic hazard:
The reported LC50 (96h) from the acute fish toxicity study (Danio rerio; Anonymous (2016)) is 0.87 mg/L based on measured concentrations. Could you please clarify how this LC50 was derived?
Based on the information available, we do not come to the same conclusion considering this LC50. The information found considering this study is compiled in a table in an attachment to this comment. Based on this information, there is 0% mortality at 0.89 mg/L (measured concentration). It is therefore unclear how LC50 (96h) could be set to 0.87 mg/L.

If the LC50 (96h) of 0.87 mg/L for fish is incorrect, the proposal for environmental classification (acute and chronic) needs to be reconsidered. ECHA note – An attachment was submitted with the comment above. Refer to public attachment COM_CLH_PC_TMPTA_SE_attachment.docx
Dossier Submitter's Response
FR: Thank you for your comment. There is indeed a mistake in the table from the CLH report Annex I (p84). The percentage of mortality observed at the measured concentration 0.89 mg/L was not 0% as indicated in the table but 57.1% as verified in the study report. Then, the probit analysis using linear max. likelihood regression gave a a LC_{50} of 0.87 mg/L.
RAC's response
RAC agrees with the DS response.

PUBLIC ATTACHMENTS
1. TMPTA_PARAD_Comments_PublCons_October 2019.zip [Please refer to comment No. 2, 7, 12, 15]
2. COM_CLH_PC_TMPTA_SE_attachment.docx [Please refer to comment No. 17]
3. Fowler TMPTA CLH comments.pdf [Please refer to comment No. 11]
4. Comments on the CLH report for TMPTA final.docx [Please refer to comment No. 10]