SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate (Trimethylolpropane triacrylate)

EC No 239-701-3

CAS No 15625-89-5

Evaluating Member State(s): FRANCE

Dated: 12 July 2019

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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2014

Before concluding the substance evaluation a Decision to request further information was issued on: 06 July 2016

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate identified in this document by its synonyme name Trimethylolpropane triacrylate (TMPTA) was originally selected for substance evaluation in order to clarify concerns about:

- Sensitiser
- Exposure of workers
- High RCR
- Wide dispersive use

During the evaluation also other concerns were identified. The additional concerns were:

- Carcinogenicity
- Genotoxicity
- Toxicity and risks for the environment

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A comprehensive CCH has been performed by ECHA on TMPTA and a decision was adopted on 16 December 2014. The decision[2] required the lead registrant to submit the following information by December 2015:

1. Pre-natal developmental toxicity study (Annex X, 8.7.2.; test method: EU B.31./OECD 414) in rabbits, oral route;
2. Chemical Safety report with:
    a. Revised DNELs for workers and the general population (Annex I, Section 1.4.1.);
    b. Revised predicted no effects levels (PNECs) for sediment and soil (Annex I, 3.3.1.);
    c. Revised environmental exposure assessment and risk characterisation (Annex I, sections 5 and 6);
    d. Documentation for the recommended personal protective equipment (Annex I, 5.1.1. in conjunction with Annex II, 0.1.2. and 8.2.2.2(b)).

This CCH is now concluded.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

[2] https://echa.europa.eu/documents/10162/27b0c1bf-baf3-d845-7733-d9c99ae45154

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling	x
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	
Other action: - Compliance check	X

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

The FR-MSCA considers that the current EU harmonised classification of TMPTA needs to be updated for the following endpoints:

- Add Carc. 2 – H351
- Add Aquatic Acute 1, H400 (M Factor 1); Aquatic Chronic 1, H410 (M factor 1)

Even if the available results are not considered to fullfill criteria to classify TMPTA as a germ cell mutagen agent, genotoxicity data are included in the CLH report to be discussed at the RAC level.

A respective proposal for a harmonised classification update was submitted to ECHA in 2019.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not relevant: TMPTA does not present properties relevant for SVHC identification at this stage.

4.1.3. Restriction

Not relevant: risks have not been identified in the course of this SEv.

4.1.4. Other EU-wide regulatory risk management measures

Not relevant.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not relevant.

5.2. Other actions

Compliance check (CCH):

For the fertility endpoint, there is only a screening OECD 422 assay available in the registration dossier. However, it is not an alternative to, nor does it replace the existing test Guidelines 443, as a standard requirement set in Annex X, Section 8.7.3. In this context, e-MSCA recommends ECHA to consider this substance for prioritization for CCH on this endpoint to check if further data are needed with regard to REACH requirements.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Proposal for harmonised classification update	Submitted in February 2019	France

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Trimethylolpropane triacrylate (TMPTA) was originally selected for substance evaluation in order to clarify concerns about:

- Sensitisation
- Exposure of workers
- High RCR
- Wide dispersive use

During the evaluation also other concerns were identified. The additional concerns were:

- Carcinogenicity
- Genotoxicity
- Toxicity and risks for the environment

Table 3

EVALUATED ENDPOINTS Endpoint evaluated	Outcome/conclusion
Acute toxicity	No concern identified.
Corrosion / irritation	No further action
Skin / respiratory sensitisation	Skin sensitization: no further action. The substance is already classified as Skin Sens. 1 according to CLP regulation. Respiratory sensitisation: concern identified for SEv but clarified.
Repeated-dose toxicity	No further action.
Genotoxicity	Concern identified during Substance evaluation process. *In vivo* Comet assay provided by the registrants according to the final decision. Genotoxicity data are included in the CLH report submitted by e-MSCA in February 2019, including a proposal for classification of the substance for carcinogenicity.
Carcinogenicity	CLH process to update the current EU harmonised classification and labelling initiated: Add Carc. 2

Toxicity to reproduction	Fertility: e-MSCA recommends ECHA to to consider this substance in prioritisation for CCH, in the light of the new data, to check if further data are needed with regard to REACH requirements. Developmental toxicity: no further action.
Environment Aquatic toxicity	Concern identified during Substance evaluation process. Fish acute toxicity test was provided by the registrants according to the final decision. It was used to determine the PNEC in surface water and led to the need to classify the substance for its acute and chronic aquatic toxicity. CLH process to update the harmonized classification and labelling initiated: add Aquatic Acute 1, H400 (M factor 1) and Aquatic Chronic 1 H410 (M factor 1)
Environment Bioaccumulation potential and risk of secondary poisoning	Concern identified during Substance evaluation process. A BCF value was estimated using CATALOGIC v5.13.1 based on the Kow of 4.35. The prediction is inside the applicability domain of the model, and the estimated BCF of TMPTA is 4.26 L/Kg (log BCF = 0.63). Absence of risk of secondary poisoning was confirmed. No further action.

7.2. Procedure

The initial phase of evaluation (March 2014-March 2015) was based on the updated registration dossiers aggregated by ECHA on 19 March 2014. In addition, data from published literature were considered.

At the end of the initial phase of evaluation, concerns for genotoxicity and for environment were identified and cannot be clarified based on available data. A decision was sent to Registrants on 6 July 2016. The decision required registrants to submit the following information:

1. *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;
2. Detailed description and justifications for each contributing scenarios and revision of spraying scenarios with appropriate models;
3. Fish, Acute Toxicity Test (test method: OECD 203);
4. Evaluation of bioaccumulation potential:

- Refinement estimation of log Kow based on appropriate determination of CMC and solubility of TMPTA in octanol;
- If refined log Kow ≥3, update of secondary poisoning risk assessment based on QSAR evaluation of the bioaccumulation potential with appropriate justification and documentation that the approach is valid for TMPTA;
- If not technically possible to refine the log Kow, or if risk of secondary poisoning is identified further to risk assessment update, Bioaccumulation in Fish: Aqueous and Dietary Exposure (OECD TG 305).

Registrants were asked to update their registration dossiers with the required information before 13 October 2017.

The second phase of evaluation was started on 1 December 2017. The initial evaluation was updated to consider information provided in response to the SEv decision as well as information provided in response to the CCH decision (see section 2). This final report is based on the registration dossiers on 15 March 2018.

7.3. Identity of the substance

The substance Trimethylolpropane triacrylate (TMPTA) is a mono constituent substance (origin: organic) having the following characteristics and physico–chemical properties (see the IUCLID dataset for further details).

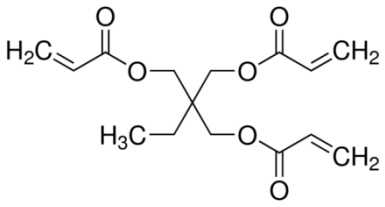
The following public name is used: Trimethylolpropane triacrylate (TMPTA).

Table 4

SUBSTANCE IDENTITY	
Public name:	Trimethylolpropane triacrylate (TMPTA)
EC number:	239-701-3
CAS number:	15625-89-5
Index number in Annex VI of the CLP Regulation:	607-111-00-9
Molecular formula:	$C_{15}H_{20}O_6$
Molecular weight range:	296.3157
Synonyms:	2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



The compositions submitted by the registrants are considered as monoconstituent according to REACH guidance for identification and naming of substances (further details in confidential annex).

Different manufacturing processes exist. They are based on the same chemical reaction but conditions (initiation, pressure, temperature…) and reactants differ. Depending on whether a purification step is performed or not, there are different impurity profiles, which lead to different classifications of the substance.

Analytical information is provided (UV/VIS, IR, NMR and GC chromatograms) to confirm the compositions and the structure of substances of each registrants.

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	***Value used for SEV:*** Clear liquid at 20°C and 1 atm
Melting / Freezing point	***Value used for SEV:*** Melting point < -20°C at 1 atm *Melting point was determined in accordance with the test method OECD Guideline 102 "Melting Point/Melting Range".*
Boiling point	***Value used for SEV:*** Boiling point >390°C at 1 atm *Boiling point was determined in accordance with the test method OECD Guideline 103 "Boiling Point".*
Relative density	***Value used for SEV:*** 1.1086 at 20°C *Relative density was determined according to the test procedure OECD Guideline 109 "Density of Liquids and Solids". Relative Density was determined at 20 °C by using the oscillating densitimeter.*
Granulometry	***Value used for SEV:*** Not relevant *TMPTA is a liquid*
Vapour pressure	***Value used for SEV:*** 0.1 Pa at 20°C *Vapour* pressure *was determined according to the test procedure OECD Guideline 104 "Vapour Pressure" (Grain-Watson estimation).*
Partition coefficient n-octanol/water (Log Kow)	***Value used for SEV:*** Log Kow (Pow): 4.35 at 25°C *Due to its surface active properties, the partition coefficient n-octanol/water of the test item can only be estimated from the single solubilities in n-octanol and in water.* *According to guidance, a working approach for surfactants might be the comparison of measured solubilities in octanol and water. However, it would then be prudent to take the critical micelle concentration in water (CMC) as a solubility limit, in order to avoid the artefact of unrealistically low Kow values*.

	Kow was calculated as the ratio between the test substance solubility in octanol and the CMC. Log Kow is 4.35 at 25°C
Water solubility	***Value used for SEV:*** 0.5 g/L at 20°C *Water solubility was determined according to the test procedure EU test method A.6 (flask method).*
Surface tension	***Value used for SEV:*** 51 mN/m at 20°C The test item is surface-active. *The surface tension was determined according to OECD Guideline 115 "Surface Tension of Aqueous Solutions". The surface tension of an aqueous solution of the test item (90% saturation concentration) at 20°C was found to be 51 mN/m. The estimated accuracy is ± 1 mN/m.* *The test item is surface-active.*
Flash point	***Value used for SEV:*** 194.5 °C at 1 atm *The flash point of the substance was determined in accordance with the test method A.9 "Flash Point" (by means of Pensky-Martens apparatus, according to DIN EN ISO 2719).*
Autoflammability / self-ignition temperature	***Value used for SEV:*** 385°C at 1 atm *The auto-ignition temperature was determined according to the test method A.15 "Auto-ignition temperature (liquids and gases)".*
Flammability	***Value used for SEV:*** Non flammable *The flammability was determined according the test method A.12 "Flammability (contact with water)".* *In the course of water solubility study according to EU A.6, it was realized that the registered substance can be mixed in water without development of gas. As no gas is developed when the registered substance gets in contact with water, the determination of the Flammability (EEC. A.12 (Contact with water)) is not applicable.*
Explosive properties	***Value used for SEV:*** Non explosive *There are no chemical groups associated with explosive properties present in the molecule, thus according to REACh legislation, Annex VII, 7.11, column 2, the study does not need to be conducted.*
Oxidising properties	***Value used for SEV:*** Non oxidizing *Based on the chemical structure the substance is incapable of reacting exothermically with combustible materials. According to REACh legislation, Annex VII, 7.13, column 2, the study does not need to be conducted.*

Stability in organic solvents and identity of relevant degradation products	Stable in organic solvents *In accordance with Column 2 of Annex IX a test on the stability in organic solvents is not necessary because this stability is not considered to be critical based on chemical structure and experience in use.*
Dissociation constant	***Value used for SEV:*** The substance does not dissociate in water. *Dissociation constant in water of the substance was determined according to OECD Guideline 112 "Dissociation Constants in Water". Dissociation constant in water was determined at 20 DC by using the conductimetric method.* *No conductivity could be measured which was ascribable on dissociated parts of the test item. Even at a concentration of 1.2 g/L, the measured conductivity is similar to the conductivity of water. Due to this fact the substance does not dissociate in water.*
Viscosity	***Value used for SEV:*** Viscosity at 20°C: 122 mPa.s (dynamic) *Viscosity of the substance was determined according to OECD Guideline 114 "Viscosity of Liquids". Dynamic Viscosity η was determined at 20 °C by using the rotational viscometer.*

No further action is required for these endpoints in the framework of this SEv.

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)			
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t
☒ 10,000-100,000 t	☐ 100,000 – 1,000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 7

USES	Use(s)
Uses as intermediate	Technical function of the substance as intermediate during formulation
Formulation	Formulation of preparations (Industrial formulation, blending, repacking in dry process: all coatings and inks) ERC2 PROC 1, 2, 3, 5, 8a, 8b, 9, 15 Substance supplied as such and in a mixture
Uses at industrial sites	Industrial application of all coatings and inks in dry process ERC5 PROC1, 2, 5, 8a, 8b, 10, 13, 15, 3 Substance supplied as such and in a mixture Industrial use in polymerisation in the polymer industry ERC6d PROC1, 3, 4, 8a, 8b, 15 Substance supplied as such and in a mixture
Uses by professional workers	Professional indoor printing with ink cartridges in dry process ERC8c PROC1, 3, 10
Consumer Uses	Use advised against
Article service life	Yes (in case of professional use)

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
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		Note D

7.6.2. Self-classification

- In the registration(s)*:* in addition to the harmonised EU classification
  - Aquatic Acute 1 – H400; M factor = 1
  - Aquatic Chronic 1 – H410; M factor = 1

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*
  - STOT SE 3 – H335
  - Aquatic Chronic 2 – H411
  - Aquatic Chronic 3 – H412

7.7. Environmental fate properties

7.7.1. Degradation

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

The biodegradability of 20 mg/L of TMPTA by microorganisms from the activated sludge of a municipal sewage treatment plant was investigated according to OECD test guideline 301B under aerobic static exposure conditions (Unpublished study report 1, 2010). The biodegradability - based on CO_2 evolution - of the test substance was calculated to be 86% of the theoretical value ($ThCO_2$) after an incubation time of 28 days and reached 66% at the end of the 10-d window. Significant biodegradation of the test substance was observed after a lag phase of about 7 days. The positive control, sodium benzoate, reached 100% biodegradation after 14 days, thus confirming suitability of inoculum and test conditions. The test substance reached the pass level of 60% for ready biodegradability in the CO_2 Evolution Test (OECD 301B) within the 10-d window and, therefore, TMPTA can be termed as readily biodegradable.

7.7.2. Environmental distribution

Based on the log Kow of 4.35, the adsorption coefficient log Koc of the test substance was estimated to be 3.2 , i. e. Koc = 1585 L/Kg (KOCWIN v2.00). This estimation indicates potentially high adsorption potential of TMPTA on organic particles (log Koc>3).

7.7.3. Bioaccumulation

A BCF value was estimated based on the Kow of 4.35 using OASIS CATALOGIC BCF base-line model v5.13., which incorporate substances with acrylate fragments. The substance falls within the parametric domain of the model (log Kow, molecular weight, water solubility), as well as within its structural domain (85.71% of the fragments are recognised as correct). The prediction is inside the applicability domain of the model, and the estimated BCF of TMPTA is 4.26 L/Kg (log BCF = 0.63). These results show that TMPTA is a low bioaccumulative substance.

No further action is required in the framework of this SEv on the environmental fate properties.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

The results are summarised in the following table:

Table 9

Method	Results	Remarks	Reference
Danio rerio Semi static OECD 203 GLP	LC_{50} (96 h): 0.87 mg/L test mat. (measured geom. mean) based on: mortality	1 (reliable) Key study Test material (common name): Trimethylolpropa ntriacrylate	Unpublished study report 2 (2016)
Leuciscus idus freshwater static EU Method C.1 (Acute Toxicity for Fish) (DIN 38412/15)	LC_{50} (96 h): 1.47 mg/L test mat. (nominal) based on: mortality	3 (not reliable) Supportive study experimental result Test material (common	Unpublished study report 3 (1988)

Method	Results	Remarks	Reference
Not GLP		name): Trimethylolpropantriacrylate	

Two acute toxicity studies of TMPTA on fish are available. In the first key study, TMPTA toxicity on *Danio rerio* under semi-static conditions for 96h was assessed according to the OECD 203 Guideline. Fish were exposed to a series of test solutions renewed every day throughout the test period. Chemical analysis of the test item throughout the test period have shown instability of the substance. Therefore, the exposure concentrations were based on the geometric mean of measured concentrations 0.19, 0.41, 0.89, 1.71 and 3.10 mg/L. The 96h LC_{50} of TMPTA for the *Danio rerio* is 0.87 mg/L (measured concentration). This study followed the good laboratory practices and fulfilled all validity criteria.
In the *Leuciscus idus* study, the results showed that no mortality occurred at the first four concentrations (0.1, 0.215, 0.464 and 1 mg/L) whereas 100% of fish died at the highest tested concentration of 2.15 mg/L. These results are not consistent with the results observed in the two range-finding studies mentioned in the Study report (Unpublished study report 3, 1988) where LC_{50} between 0.3 and 1 mg/L were detected. The LC_{50} might therefore be below 1 and there is a high uncertainty on the data provided. Besides these questionable toxicity results, no concentrations were measured in this static acute study performed on a surface-active substance and the toxic effect relates to the nominal concentration of TMPTA. Consequently, this acute study on fish is not considered reliable and is used only as supportive data.

7.8.1.2. Aquatic invertebrates

Two acute toxicity studies on daphnia are available (Unpublished study report 4, 1991; Unpublished study report 5, 1988). Results are used as supportive data since some information in these study reports could not be verified (GLP conditions, no analytical measures). *D. magna* were exposed for 48h in a static system. Based on these two studies, TMPTA is considered to be moderately toxic to daphnia with CE_{50} = 19.9 mg/L (nominal concentration), and aquatic invertabrates are less sensitive than fish.

7.8.1.3. Algae and aquatic plants

The test substance was tested for aquatic toxicity to the algae *Scenedesmus subspicatus* according to the method DIN 38412/9 (Unpublished study report 6, 1989). Results are used as supportive data since some information in these study reports could not be verified (GLP conditions, no analytical measures). After 96h exposure the aquatic toxicity was determined to be: ErC_{10} = 2.18 mg/L and ErC_{50}=14.5 mg/L (nominal concentration). TMPTA is considered to be moderately toxic to algae which are less sensitive than fish.

7.8.1.4. Sediment organisms

According to Annex X of Regulation (EC) No 1907/2006, long-term toxicity tests for sediment organisms data are not needed, as the results of the chemical safety assessment does not indicate the need to investigate further the effects of the substance and/or relevant degradation products on sediment organisms. Then, the equilibrium partitioning method is used for assessing the hazard to sediment organisms.

7.8.1.5. Other aquatic organisms

No relevant information available

Overall, from available data, fish is identified as the most sensitive aquatic species and the 96h LC_{50} for the *Danio rerio* of 0.87 mg/L can be used to derive the aquatic PNEC.

No further action is required in the framework of this SEv.

7.8.2. Terrestrial compartment

According to Annex X of Regulation (EC) No 1907/2006, chronic toxicity tests for terrestrial organisms are not needed, since the results of the chemical safety assessment does not indicate the need to investigate further the effects of the substance and/or relevant degradation products on soil organisms. Then, the equilibrium partitioning method is used for assessing the hazard to soil organisms.

No further action is required in the framework of this SEv.

7.8.3. Microbiological activity in sewage treatment systems

Relevant studies on the toxicity of TMPTA to STP microorganisms are not available. Consequently, the results from the biodegradation study (see 7.7.1) are used to derive the $PNEC_{STP}$. In this study, the biodegradation rate of the inoculum has not been affected at 20 mg TMPTA/L. Therefore, a NOEC of 20 mg/L for STP microorganisms (nominal concentration) is used for assessing the hazard to microorganisms in sewage treatment plants.

No further action is required in the framework of this SEv.

7.8.4. PNEC derivation and other hazard conclusions

Table 10

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC freshwater = 8.7E-04 mg/L	Lowest LC_{50} for fish of 0.87 mg/L (measured concentration) with the assessment factor: 1000
Marine water	PNEC marinewater = 8.7E-05 mg/L	Lowest LC_{50} for fish of 0.87 mg/L (measured concentration) with the assessment factor: 10000
Intermittent releases to water	PNEC freshwater = 8.7E-03 mg/L	According to the guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation

		of dose [concentration]-response for environment (ECHA 2008), if intermittent release is identified for a stage of the life cycle, only short-term effects need to be considered for risk characterisation of that stage (only for the aquatic compartment); in this case, the assessment factor can be reduced from 1000 to 100.
Sediments (freshwater)	PNEC sediment = 1.4E-01 mg/kg dw (3.07E-02 mg/kg ww)	No data on sediment organisms is available. According to the Guidance on information requirements and chemical safety assessment – Chapter R.10: Characterisation of dose [concentration]-response for environment, in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNECsed may be provisionally calculated using the equilibrium partitioning method (EPM). This method uses the PNECwater for aquatic organisms and the suspended matter/water partitioning coefficient as inputs. The following formula can therefore be applied: PNECsed = (Ksusp-water / RHOsusp) * PNECaqua * 1000 with : RHOsusp : bulk density of wet suspended matter = 1150 kg/m^3 and Ksusp-water = partition coefficient suspended matter water = 40.52 m^3/m^3 (estimated from the equation R16.7 of the guidance document of ECHA (2010) and an estimated logKoc value of 3.2 using the equation for non hydrophobic substances in the TGD (EC, 2003)). PNEC has been recalculated to dry sediment: PNEC sediment dry = PNEC sediment wet * 4.6.
Sediments (marine water)	PNEC sediment = 1.4E-02 mg/kg dw (3.07E-03 mg/kg ww)	No data on sediment organisms is available. According to the Guidance on information requirements and chemical safety assessment – Chapter R.10: Characterisation of dose [concentration]-response for environment, in the absence of any ecotoxicological data for sediment-dwelling organisms, the PNECmarine sediment may provisionally be calculated using the equilibrium partitioning method. This method uses the

		PNECsaltwater for aquatic organisms and the marine suspended matter/water partitioning coefficient. This method results in a PNEC value of 10 times lower than the PNEC value for freshwater sediment.
Sewage treatment plant	PNECstp = 2 mg/L	No reliable study assessing the toxicity of the registered substance to microorganisms is available. Based on the biodegradation study where no adverse effect on STP micro-organisms is expected up to 20 mg/L. To this value the assessment factor of 10 was applied to derive the PNECstp.
Soil	PNEC soil = 2.76E-02 mg/kg dw (2.44E-02 mg/kg ww	No data on soil organisms is available. According to the Guidance on information requirements and chemical safety assessment – Chapter R.10: Characterisation of dose [concentration]-response for environment, in the absence of any ecotoxicological data for soil organisms, the PNECsoil may be provisionally calculated using the equilibrium partitioning method (EPM). This method uses the PNECwater for aquatic organisms and the soil/water partitioning coefficient as inputs. The following formula can therefore be applied: PNECsoil = (Ksoil-water / RHOsoil) * PNECaqua * 1000 with : RHOsoil: bulk density of wet soil = 1700 kg/m^3 and Ksoil-water = partition coefficient suspended matter water = 47.8 m^3/m^3 (estimated from the equation R16.7 of the guidance document of ECHA (2010) and an estimated logKoc value of 3.2. PNEC has been recalculated to dry soil: PNEC soil dry = PNEC soil wet * 1.13.
Air	-	-
Secondary poisoning	No potential for bioaccumulation	Low BCF value of 4.26

7.8.5. Conclusions for classification and labelling

Based on the lowest aquatic acute toxicity value lower than 1 mg/L (96h LC_{50}=0.87 mg/L) and the absence of aquatic chronic data, TMPTA needs to be classified as aquatic acute category 1 H400 (M-Factor = 1) and aquatic chronic category 1 H410 (M-Factor=1) according to the Regulation (EC) No 1272/2008.

This endpoint is included in the CLH report that France submitted in February 2019.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Oral and GI absorption:

No experimental data are available with TMPTA regarding oral absorption. Following the REACH guidance document 7c, the physicochemical properties of TMPTA (molecular weight of ~296 g/mol and water solubility of 500 mg/L) are favourable to oral absorption. According to Danish QSAR database, an absorption from gastro intestinal tract for a dose of 1 mg is estimated at 95% and for a dose of 1000 mg at 50%. Additionally, acute oral toxicity studies (Unpublished study report 7, 1972; Unpublished study report 8, 1980) showed deaths indicating some evidence of bioavailability. Finally, considering the irritating properties of TMPTA, oral absorption may be enhanced by irritation of the gastro-intestinal tract.

Inhalation absorption:

No experimental data are available with TMPTA regarding inhalative absorption. According to the REACH guidance document 7c, physicochemical data enable qualitative judgments of the toxicokinetic behavior. The limited vapour pressure and water solubility property of the substance are not in favour of respiratory absorption. Furthermore, the result of acute inhalation toxicity studies shows no toxicity up to the vapour saturation concentration (Unpublished study report 9, 1976) indicating either a low absorption of TMPTA and/or a low toxicity potential after inhalation.

Dermal absorption:

An *in vivo* study was performed in rats and mice (NTP, 2005). This study shows an inverse dose dependent dermal absorption rate. It is shown that a total of 18.7% of the 130 mg/kg dose, 32.7% of 15.2 mg/kg dose and 55.1% of 1.7 mg/kg dose were absorbed in rats after a single dermal application. Due to the irritative potential of the substance, it may also be possible that absorption increased after repeated exposure to the substance. When rats were pre-exposed to 151 mg/kg bw of non-radiolabeled TMPTA 24h prior to the same dose of radiolabeled TMPTA, the dermal absorption was 25.4%. This confirms that repeated dose exposure of TMPTA can enhance dermal absorption (25.4% absorbed after 2 applications of 151 mg/kg vs 18.7% after single application of 130 mg/kg). Total recoveries ranged from 89.7 to 94.8% which is lower than the minimal recovery (95%) recommended by EFSA guidance (2017). Therefore, the actual absorption may be underestimated based on this study in rats. In mice dermally exposed to 1.2 mg/kg, 75% of TMPTA was absorbed. The total recovery was 95.9%. In conclusion, significant amounts of TMPTA are absorbed if applied dermally in rats and mice. Dermal absorption is higher in mice compared to rats.

A recent *in vitro* percutaneous absorption study through human skin (Unpublished study report 10, 2015) was included in the latest update of the registration dossier (September 2017). Breast skins were exposed to TMPTA (890 g/L) for 8 hours. Samples used to estimate dermal absorption were collected until 24 hours after application. The authors concluded to an absorption of the neat substance at 0.60% when considering the amount in the receptor fluid, the receptor compartment wash, the skin membrane and the stratum corneum excluding the first 2 tape strips. According to EFSA guidance (EFSA, 2017), a multiple of the standard deviation should be added to the mean dermal absorption value leading to a dermal absorption of 0.8%. Only one high non-diluted concentration was used in this study. Without any detailed information on the typical formulations on the market, it is not possible to assess the relevance of the obtained dermal value from this study to the expected use conditions.

Distribution and accumulative potential:

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

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

Metabolism:

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

Reactivity:

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

Excretion:

Based on the physico-chemical information (molecular weight and water solubility), main excretion via kidney can be expected. In addition, based on the suspected degradation of TMPTA to acrylic acid and the known degradation of acrylic acid to CO_2, exhalation is also expected to be a significant route of excretion. These major routes of excretion are confirmed within the NTP (2005) study. After IV administration in rats, [C^{14}]-TMPTA was mainly measured in urine (48%), then in expired CO_2 (20.1%) and faeces (8.7%). After dermal application in rats, the major route of elimination was also the urine (3-28%), followed by expired CO_2 (1.4-13%) and faeces (0.2-2.5%). Excretion was dose-dependent, with higher elimination rate after lower doses tested. In mice, TMPTA was eliminated at similar amount in urine and expired CO_2 (16-18%) and then in faeces (5.6%). In sum, based on amounts found in urine, cage wash, exhaled air and faeces, the total radioactivity excreted in 72h was found to be between 4.7% (130 mg/kg bw) to 45% (1.7 mg/kg bw) in rats exposed dermally or 84% after IV administration.

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity:

Based on the LD_{50} (rat) for acute oral (Unpublished study report 7, 1972; Unpublished study report 8 (1980)) and dermal (Unpublished study report 7, 1972; Unpublished study report 11, 1981) toxicities (above 2000 mg/kg bw), TMPTA is not acutely toxic by these routes of exposure. Although only studies by inhalation of limited quality are available (Unpublished report 12, 1980; Unpublished study report 9, 1976), TMPTA is considered of low toxicity due to its low volatility and the absence of mortality at saturation concentration.

Irritation:

TMPTA is currently classified as Skin Irrit. Cat 2 – H315 (CLP00), this classification corresponding to a conversion from the classification set according to 67/548/EEC Directive.

Three among the 10 studies available lead to a classification as Skin irritant (Xi, R38) according to 67/548/EEC Directive: Unpublished study report 13 (1978), Unpublished study report 14 (1978); Unpublished study report 15 (1977) and Unpublished study report 16 (1990) (mean score >= 2 for erythema and/or oedema). According to CLP regulation, only results from Scibor (1977) fulfill criterion for Skin Irrit. 2 – H315 (mean score >= 2.3 - <= 4.0 for erythema and/or oedema) after an exposure to the tested substance for 24 hours instead of 4 hours as recommended in OECD guideline 404. Local skin irritation (including hyperplasia and chronic inflammation) was also reported in the available repeated-dose toxicity studies by dermal route (NTP (2005) & (2012)). Based on all these data, the current harmonized EU classification is considered justified. No further action is required for this endpoint in the framework of this SEv.

Clear irritating effects on rabbit's eye were observed in 2 reliable eye irritation studies performed with TMPTA (not further characterized) (Unpublished reports 13 and 14 (1978). The mean scores (24, 48, 72h) obtained are in line with the classification as Eye irrit. cat. 2 – H318 but reversibility was not obtained within the 7 days post-exposure. In particular, corneal damage increased during the post treatment period. In 2018, a bovine corneal opacity and permeability test (BCOP test) was carried out with TMPTA (purity = 80.2%) (Unpublished study report 17, 2018). After a topical application of the neat substance for 10 minutes, a mean *in vitro* irritancy score (IVIS) of 0.9 was obtained. The IVIS is below the threshold of 3 set in the OECD guideline 437, resulting of no classification based on this study.

TMPTA is currently classified as Eye Irrit. 2 according to CLP regulation. Based on the *in vivo* eye irritation studies, it can be questioned if a more stringent classification as Eye Dam. 1 – H318 is required based on the non-reversibility of the effects. However, the substance is not classified as Skin Corr and the current harmonized classification is based on the studies performed in 1978 that show an absence of reversibility at the observation time. Considering the results of the recent BCOP test, it is considered that the current harmonized classification Eye Irrit. 2 is appropriate.

Due to its low volatility and the absence of symptoms in the acute inhalation studies, it is unlikely that TMPTA is a respiratory irritating agent. No further action is required in the framework of this SEv.

7.9.3. Sensitisation

Skin sensitisation

- *Experimental data*

TMPTA was tested for its potential as a skin sensitizer in experimental assays using different methodologies.

Skin sensitisation has been observed in two maximisation tests in guinea pig. In the first assay (Nethercott *et al.* 1983), 4/20 guinea pigs that were administered 0.5% TMPTA and 10/20 administered a 10% solution became sensitized. In the second assay (Björkner, 1980), 6/24 guinea pigs exposed to 0.1% and 16/24 exposed to 0.5% became sensitized. No cross sensitisation to trimethylol propane trimethacrylate (TMPTMA) or acrylic acid could be observed.

TMPTA also induced skin sensitization in mice exposed to 0.1% of TMPTA in a LLNA (local lymph node assay) and to 0.3% in a MEST (mouse ear swelling test)(Hayes & Meade (1999)). Irritation was reported at tested concentrations from 1.0% in the irritancy test. Cross-reactivity was also assessed by Hayes & Meade (1999) in a MEST. Cross-reactivity was seen when the animals were sensitized with TMPTA and challenged with n-butyl acrylate.

In contrast, other experimental studies show no skin sensitisation after administration to TMPTA. However, these studies did not specify if a positive control was included to validate the results (MEST and LLNA conducted by the National Toxicology Program (NTP, 2005)) or present numerous discrepancies during an inspection according to the registrants (modified guinea-pig Buehler test performed by Unpublished study report 18, 1984).

- *Human data*

There are several cases of skin sensitization in human exposed to TMPTA (Emmett *et al*., 1977; Björkner *et al.,* 1980; Nethercott *et al*., 1978 & 1983; Dahlquist *et al*., 1983; Kanerva *et al*., 1998, Christoffers et al., 2013), most of them occurring at workplace and especially among workers exposed to ultraviolet printing inks. Since workers can be exposed to mixture of acrylates, either cross-sensitization or concomitant sensitization may be possible. In particular, some publications suggest a cross-sensitization between TMPTA and PETA (pentaerythritol triacrylate) (Dahlquist *et al.,* 1983; Cofield *et al.,* 1985).

Cases of allergic conjunctivitis due to occupational exposure to TMPTA were also reported by Kanerva *et al*., 1998 and Mancuso & Berdondini, 2008.

In conclusion, skin sensitization induced by TMPTA is observed in both experimental animals and humans. TMPTA is currently classified as Skin Sens. 1 – H317 according to CLP regulation. It has been investigated during SEv if a subcategory can be proposed. However, conflicting results were obtained from Maximisation studies since criteria were fulfilled for a category 1A from the Björkner (1980) study but only a category 1B from the Nethercott *et al.* (1983) study. From human data, the lack of information on exposure does not allow to reach a firm conclusion on a subcategory. In this context, no subcategory is proposed for skin sensitization. No further action is required for this endpoint in the framework of this SEv.

Respiratory sensitisation

Some animal and non animal test methods for the identification of respiratory sensitisers have been described in the literature, but these are not widely accepted yet, nor close to the point where they could enter formal validation.

In this context, the RIVM (personal communication) has run different SAR models (Derek, Jarvis, CatSAR, Enoch, MultiCase) with acrylates in 2014. The predictions for acrylates differ greatly between the SARs used. Indeed, for TMPTA, Jarvis and Enoch predicted a respiratory sensitisation potential; Derek and CatSAR, no respiratory sensitisation potential and MultiCase gave no prediction. Similarly, according to Danish (Q)SAR database (2018), the prediction is outside the applicability domain for CASE Ultra and SciQSAR and negative

in Leadscope model. Therefore, no reliable conclusion can be reached for this substance based on (Q)SAR models.

One case of asthma was reported in the literature (Sanchez-Garcia, 2009). This concerns a nonsmoking 62-year-old woman without atopy in whom asthma symptoms developed after she had worked for 20 years selling lottery tickets inside a 4-m^3 kiosk. This individual became asymptomatic when exposure to the compound containing TMPTA ceased. Bronchial challenge tests were performed showing decreased FEV_1 (forced expiratory volume in one second), increased eosinophil count in sputum and elevated FE_{NO} (fraction of nitric oxide in exhaled air) after challenge with TMPTA.

In France, a national occupational disease surveillance and prevention network (RNV3P) created in 2001 collects every year more than 8000 new occupational health reports throughout France. From this database, no clinical case related to respiratory sensitisation and specifically related to TMPTA has been reported in Occupational disease consultation centres (CCPP) during the period 2001-2017 (RNV3P database, 2018).

In summary, no relevant alert was found from SAR models and only one human case of respiratory sensitisation was reported in the literature for TMPTA. Furthermore, considering the limited exposure potential by inhalation due to the low vapour pressure, TMTPA was considered of low respiratory sensitisation potential. No further action is required for this endpoint in this SEv framework.

7.9.4. Repeated dose toxicity

7.9.4.1. Repeated dose toxicity: oral

The results of studies on repeated dose toxicity after oral administration are summarised in the following table:

Table 11. Studies on repeated dose toxicity after oral administration

Method	Results	Remarks	Reference
14-day dose range finding study for combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of TMPTA in rats by oral route Wistar Han rats (5/sex) Vehicle: polyethylene glycol 400 Oral gavage once daily for 14 days 0, 100, 300, 1000 mg/kg bw/day Parameters evaluated: clinical signs, body weight, food consumption, macroscopy at termination, selected organ weights	Local toxicity(forestomach and glandular mucosa of the stomach) in all animals from 300 mg/kg bw/day. Decreased body weight and body weight gain in males at 1000 mg/kg bw/day. Increased relative and absolute liver weight in females and higher relative testis weight for males at 1000 mg/kg bw/day.	2 (reliable with restriction) experimental result **Test material (EC name): TMPTA** Purity = 80.2%	Unpublished study report 19 (2015)

Method	Results	Remarks	Reference
(heart, kidneys, liver, ovaries, testes, spleen). Non GLP, non-guideline			
Combined 28 day repeated dose toxicity study with the reproduction/developmental toxicity screening test Wistar Han rats (10/sex) Vehicle: polyethylene glycol 400 Oral gavage Males exposed for 29 days (beginning 2 weeks prior mating). Females treated for 41-55 days (2 weeks prior mating until lactation day 4). 0, 30, 100, 300 mg/kg bw/day GLP, OECD 422 (1996)	NOAEL local effect = 30 mg/kg bw/day NOAEL systemic effect, reproduction and development = 300 mg/kg bw/day	Reliability not evaluable considering the uncertainties linked to PEG 400 experimental result **Test material (EC name): TMPTA** Purity = 80.2%	Unpublished study report 20 (2015)

7.9.4.2. . Repeated dose toxicity: inhalation

No relevant information available

7.9.4.3. Repeated dose toxicity: dermal

The results of studies on repeated dose toxicity after dermal administration are summarised in the following table:

Table 12. Studies on repeated dose toxicity after dermal administration

Method	Results	Remarks	Reference
rats and mice (F344/N rats and B6C3F1 mice); 5/sex/dose 12.5 - 25 - 50 - 100 - 200 mg/kg bw (nominal per unit body weight) Vehicle: acetone	NOAEL local rats and mice (males and females) < 12.5 mg/kg bw. NOAEL systemic rats and mice ≥ 200 mg/kg bw.	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)

5 days per week for 16 days Painted solutions of TMPTA applied on the back NTP protocol.			
rats and mice (Fischer 344 rats and B6C3F1); 10/sex/dose 0.75 - 1.5 – 3 - 6 - 12 mg/kg/day (nominal per unit body weight) Vehicle: acetone 5 days per week for 14 weeks Painted solutions of TMPTA applied on the back Additional groups of 10 male and 10 female rats designated for clinical pathology testing received the same doses for 23 days NTP protocol	NOAEL systemic rats and mice ≥ 12 mg/kg bw/day NOAEL local male rats < 0.75 mg/kg bw/day (increase of epidermis hyperplasia) NOAEL local female rats = 0.75 mg/kg bw/day (hyperplasia of sebaceous gland) NOAEL local male mice = 1.5 mg/kg bw/day (hyperplasia and degeneration of epidermis, chronic active inflammation of the dermis, hyperkeratosis and hyperplasia of the sebaceous gland) NOAEL local female mice = 0.75 mg/kg bw/day (chronic active inflammation of the dermis)	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)
rabbit (New Zealand White); 5/sex/dose 500 mg/kg bw/day (nominal per unit body weight) Vehicle: unchanged Exposure: Two weeks (Daily, five days/week) Painted solutions of TMPTA applied on the back Six animals per group were sacrificed after 15 days and remaining 4 animals after 30 days. Animals were monitored for clinical signs and mortality, body weight gains, dermal reactions,	NOAEL local < 500 mg/kg bw. It is not possible to adequately set a NOAEL for systemic effects since there is not enough information on incidence and severity of the clinical signs (decreased motor activity and nasal discharge) and decreased body weight.	3 (not reliable) experimental result **Test material (EC name): TMPTA** Purity not stated	Unpublished study report 21 (1979)

gross pathology and histopathology.			

7.9.4.4. Repeated dose toxicity: other routes

No relevant information available

7.9.4.5. Human information

No relevant information available

7.9.4.6. Summary and discussion of repeated dose toxicity

Oral studies:

In the updated CSR dated on March 2018, two new studies were submitted by the registrants.

The first study consists in a 14-day study to define doses to be used in a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of TMPTA in rats by oral route (Unpublished study report 22, 2015). In this study, Wistar Han rats were administered TMPTA in PEG 400 by gavage at the doses of 0, 100, 300 or 1000 mg/kg bw/day once daily for 14 days. The following parameters were evaluated: clinical signs, body weight and food consumption, macroscopy and selected organ weights (heart, kidneys, liver, ovaries, testes and spleen). Treatment-related clinical signs included salivation (from 100 mg/kg bw/day in both sexes), occasional bleedings (from 300 mg/kg bw/day), hunched posture, piloerection, and diarrhea (at 1000 mg/kg/day in both sexes). In males of the 1000 mg/kg bw/day group, there was a slight body weight loss from days 1-5, followed with recovery, and decreased mean body weight gain throughout the duration of treatment. Slightly lower food intake (absolute and relative to body weight) was recorded for females at 1000 mg/kg/day from days 1-5, followed by complete recovery. Treatment resulted in local toxicity in the forestomach and glandular mucosa of the stomach. Irregular surface of the forestomach was observed in 2, 5 and 5 males and 1, 5 and 5 females of the 100, 300 and 1000 mg/kg/day groups, respectively. Furthermore, there were 1 low-dose male (100 mg/kg/day) and 2 high-dose males (1000 mg/kg/day) with reddish foci on the glandular mucosa of the stomach. At necropsy, in the 1000 mg/kg/day group, higher liver weights (absolute and relative to body weight) were recorded for females and higher relative testis weight for males. Based on these results, the doses selected for the definitive study were 30, 100, and 300 mg/kg bw/day.

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. There was no treatment-related toxicity on the reproduction at doses up to the highest dose level tested of 300 mg/kg bw/day. Based on this study, the NOAEL for parental generation is 30 mg/kg bw/day for local toxicity (forestomach irritation) and 300 mg/kg bw/day for systemic toxicity (no treatment-related effect). Developmental findings are described under section 7.9.7.

Even if this study follows OECD guideline, it is noted that the choice of the solvent used (PEG 400) is rather unusual. Considering that TMPTA is soluble in organic solvents, it is not clear why a more common solvent had not been used (e.g. corn oil, as recommended in the OECD guideline). In the literature, PEG 400 is reported as well tolerated in different species (Pandey *et al*., 2017; Gad *et al*., 2016; Healing *et al*., 2015; Thackaberry *et al*., 2010). However, PEG 400 is also known for its anti-inflammatory or anti-oxidant properties (Ackland *et al*., 2010; Juarez-Moreno *et al.,* 2015). In addition, some publications report interactions with other substances affecting their systemic absorption or reducing their adverse effects (Ma *et al.,* 2017; Ackland *et al*., 2010; Hodoshima *et al.*, 2004; Klugman *et al.,* 1984). In particular, pegylation is used in pharmaceutical sector in order to improve the tolerability of medicine. Considering that, PEG 400 may reduce or affect the intrinsic toxicity of TMPTA. In this context, it cannot be ruled out that in the absence of PEG 400, TMPTA may had induced effects at lower doses than the NOAELs set in the OECD guideline 422 study. More research is needed to characterize possible interferences of this solvent in toxicological studies.

Inhalation studies:

There was no repeated dose toxicity study by inhalation. However, considering the low volatility of TMPTA, inhalation is not considered a major route of exposure.

Dermal studies:

In a NTP range finding study (NTP, 2005), F344/N rats or B6C3F1 mice were administered 0, 12.5, 25, 50, 100, or 200 mg TMPTA/kg body weight/day, 5 days per week for 16 days. All rats and mice survived to the end of the study. Mean body weights of dosed rats were similar to those of the vehicle controls. In mice, the body weight gain of high dose males was significantly reduced (without impact on body weight), while female body weight was significantly increased. Irritation at the site of application was most commonly seen in rats and mice administered 50 mg/kg or greater. Microscopically, non-neoplastic lesions occurred at the site of application in all dose groups. Animals showed epidermal hyperplasia, hyperkeratosis, sebaceous gland hyperplasia, chronic active inflammation of the dermis. More severe lesions occurring generally at the higher doses were ulceration, epidermal degeneration, and parakeratosis at the site of application. Thymus weights of male mice administered 50 mg/kg bw/d or greater were significantly decreased. Histopathology detected thymic atrophy characterized by depletion of cortical lymphocytes in the two highest dose groups. Rats and female mice were not affected. Since thymus effects occurred in a context of severe dermal toxicity in male mice and were not consistently found in the NTP studies of longer duration, this effect seems rather due to stress than direct effect of the substance (Greaves, 2007). The systemic dermal NOAEL for rats and mice was ≥ 200 mg/kg bw/day. The dermal NOAEL for local effects in both species was < 12.5 mg/kg bw/day.

In the subsequent study, F344/N rats and mice were administered 0, 0.75, 1.5, 3, 6, or 12 mg TMPTA/kg body weight, 5 days per week for 14 weeks. No mortality and no difference in body weight were observed for mice and rats. Irritation at the site of application was noted at 12 mg/kg bw/day. Microscopically, epidermal hyperplasia occurred in all dosed groups of male rats. At higher doses (from 1.5 mg/kg bw/day), epidermal hyperplasia, degeneration, and necrosis (females only), chronic active inflammation of the dermis, hyperkeratosis, and sebaceous gland hyperplasia were reported in rats of both sexes at the site of application with a dose dependent increase in severity. Similarly, in mice, epidermal hyperplasia was observed at the site of application from 1.5 mg/kg bw/d in females and from 3 mg/kg bw in males. From 3 mg/kg bw/d in

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

TMPTA was also tested in a 2-year oral study in rats and mice (NTP, 2012). Results are described in section 5.8 (Carcinogenicity).

The same findings were reported in a repeated dermal toxicity study of low reliability (Unpublished study report 21, 1979). New Zealand White rabbits received topical application of 0 or 500 mg/kg bw of TMPTA to the back, once daily for 5 days per week during 2 weeks. Six animals per group were sacrificed after 15 days and the remaining 4 animals after 30 days. Evaluation of treated skin revealed severe necrosis of the epithelium and upper dermis after 15 days and epithelial and sub epithelial dermal necrosis after 30 days. Motor activity was decreased and nasal discharge occurred in several animals in the treated group. Few animals exhibited slight body weight losses. Microscopic examination of selected tissues revealed no evidence of systemic toxicity resulting from administration of TMPTA.

7.9.5. Mutagenicity

7.9.5.1. <u>In vitro data</u>

The results of *in vitro* genotoxicity studies are summarised in the following table:

Table 13. *In vitro* genotoxicity studies

Method	Results	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 20, 100, 500, 2500 and 5000 µg/plate for all strains (1st experiment) and 0-4000 µg/plate for TA1535 (2nd experiment)	Negative for TA 1537, TA98 and TA100 with metabolic activation. Cytotoxicity: slight decrease for TA 98 above 2500 µg/plate Positive (from 500 µg/plate – without clear dose-dependent relationship) for TA 1535 with metabolic activation; not cytotoxic	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA**	Unpublished study report 24 (1989)

Positive control substance(s) included Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation; not cytotoxic Negative control, vehicle control and positive controls valid.	Purity > 70%	
bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 100 – 10000 µg/plate Positive control substance(s) included Equivalent or similar to OECD Guideline 471	Negative for TA 1535, TA 1537, TA 98 and TA 100 without metabolic activation and with metabolic activation (rat S9); not cytotoxic Vehicle and positive controls valid Positive for TA 1535 with hamster S9	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 79%	Cameron et al. (1991)
bacterial reverse mutation assay (e.g. Ames test) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Test concentrations: 0.2, 2, 20, 500 µg/plate for the incorporation assay and 5 mg for the spot test. Positive control substance(s) included equivalent or similar to OECD Guideline 471	Negative for TA 1535, TA 1537, TA 98 and TA 100; with and without metabolic activation. Cytotoxicity: growth inhibition in the spot assay, not reported in the incorporation assay Vehicle, negative and positive controls valid	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity not stated	Unpublished study report 25 (1976)
bacterial reverse mutation assay (pre-incubation method) *Salmonella typhimurium* (TA98 and TA100) or *Escherichia coli* (WP2 *uvrA*/pKM101) (met. act.: with and without) Test concentrations: 1,500 to 10,000 µg/plate Positive control substance(s) included equivalent or similar to OECD Guideline 471	Negative for all tested strains. Slight toxicity at 10,000 µg/plate with S9 Vehicle and positive controls valid	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity > 78%	NTP (2012)

Gene mutation assay mouse lymphoma L5178Y cells (met. act.: with and without) Preliminary toxicity test: 0.004875 to 5 nL/mL (without S9); 0.004875 to 40 nL/mL (with S9). Mutation test: Test 1: 0.078 to 1.25 nL/mL without S9; 0.150 to 2.50 nL/mL with S9 Test 2: 0.150 to 1.00 nL/mL without S9; 1.250 to 10.00 nL/mL with S9 Test 3: 1.00 to 2.5 nL/mL without S9; 2.00 to 20 nL/mL with S9 Positive control substance(s) included equivalent or similar to OECD Guideline 476	Positive for mouse lymphoma L5178Y cells without metabolic activation; cytotoxicity: yes Unconclusive (Trial 1), negative (Trial 2), positive (Trial 3) with metabolic activation; cytotoxicity: yes Vehicle, negative and positive controls valid	2 (reliable with restriction) experimental result **Test material (EC name): TMPTA** Purity not stated	Unpublished study report 26 (1979)
Gene mutation assay Chinese hamster Ovary (CHO) (met. act.: without) Test concentrations: 0, 0.2, 0.6, 0.7 µg/mL Positive control substance included Equivalent or similar to OECD Guideline 476 Chromosomal aberrations were also examined in CHO in this publication.	Negative for gene mutation in CHO without metabolic activation; cytotoxicity: yes Vehicle and positive controls valid Positive for chromosome aberrations in CHO cells without metabolic activation	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity not stated	Moore *et al.* (1989)
Gene mutation assay mouse lymphoma L5178Y cells (met. act.: without) Test concentrations: 0, 0.6, 0.65, 0.7 µg/mL Positive control substance(s) included equivalent or similar to OECD Guideline 476	Positive (exclusive induction of small colonies) for mouse lymphoma L5178Y cells without metabolic activation ; cytotoxicity: yes Vehicle positive controls valid Positive for induction of micronucleus and	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA**	Moore *et al.* (1989) Dearfield (1989)

Induction of micronucleus and chromosome aberrations were also investigated in L5178Y cells without metabolic activation.	chromosomal aberrations in L5178Y	Purity not stated	
Gene mutation assay mouse lymphoma L5178Y cells (met. act.: with and without) Test concentrations: 0.0005 - 100µl/ml Positive control substance(s) included equivalent or similar to OECD Guideline 476	Negative for mouse lymphoma L5178Y cells with metabolic activation ; cytotoxicity: yes Vehicle and positive controls valid Positive for mouse lymphoma L5178Y cells without metabolic activation; cytotoxicity: yes Vehicle and positive controls valid	2 (reliable with restriction) experimental result **Test material (EC name): TMPTA** Purity = 79%	Cameron et al. (1991)
mammalian chromosome aberration test Lymphocytes: primary cell cultures from human peripheral blood (met. act.: with and without) Preliminary experiment: 23.1, 45.7, 92.6, 185, 370, 740, 1480 and 2960 µg/mL for the preliminary experiment both with and without S9 mix Main experiment: 1) 0.78, 1.56, 3.13, 6.25, 12.5, 18.75, 25 and 37.5 µg/mL for the first experiment without S9 mix 2) 1.56, 3.13, 6.25, 12.5, 18.75, 25, 37.5 and 50 µg/mL for the first experiment with S9 mix 3) 3.13, 6.25, 9.38, 12.5, 18.75 and 28.13 µg/mL, for the second experiment without S9 mix 4) 9.38, 18.75, 28.13, 37.5, 50 and 75 µg/mL, for the second experiment with S9 mix	Positive with and without metabolic activation Cytotoxicity: observed at all concentrations of the preliminary experiment and in both first and second experiments at conc. ≥ 12.5 µg/mL Vehicle and positive controls valid	1 (reliable without restriction) experimental result **Test material (EC name): TMPTA** Purity = 84.6%	Unpublished study report 27 (2005)

Positive control substance(s) included OECD Guideline 473 EU Method B.10 EPA OPPTS 870.5375			

7.9.5.2. In vivo data

The results of *in vivo* genotoxicity studies are summarised in the following table:

Table 14. *In vivo* genotoxicity studies

Method	Results	Remarks	Reference
micronucleus assay (chromosome aberration) mouse (Swiss Ico: OF1 (IOPS Caw)) male/female oral: gavage 437.5, 875 and 1750 mg/kg bw (for males) or 500, 1000 and 2000 mg/kg bw (for females) (nominal conc.) Positive control substance: Cyclophosphamide; 50 mg/kg bw; oral route OECD Guideline 474 EU Method B.12 EPA OPPTS 870.5395	Negative (male/female) Toxicity: only in males (piloerection at 875 mg/kg bw; 2 deaths and piloerection in surviving animal at 1750 mg/kg bw) Vehicle and positive controls valid	2 (reliable with restrictions) experimental result **Test material (EC name): TMPTA** Purity = 87.9%	Unpublished study report 28 (2006)
micronucleus assay (chromosome aberration) mouse (B6C3F) for the 14 week study Genetically modified (FVB tg.AC hemizygous) mice for the 6 month study male/female dermal 0, 0.75, 1.5, 3, 6, 12mg/kg (nominal conc.)	Negative (male/female) Toxicity: decrease in the percentage of NCEs among total erythrocytes in the 6-months-study only Vehicle controls valid: yes Positive controls valid: not included	3 (not reliable) experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)

Method	Results	Remarks	Reference
Positive control substance(s): No The study was performed as part of subchronic dermal studies described in the repeated dose section. Male and female mice were dermally exposed to the test substance 5 times per week for 14 or 28 weeks. Blood was collected from the retroorbital sinus and stained for analysis of micronuclei and NCE/PCE ratio.			
Mouse alkaline Comet assay CD-1 mice females (6/dose; 3 for positive control group) Intravenous 5, 10, 20 mg/kg; 2 doses 24h intervals; sampling 30 minutes after last dose. Organs: liver and bone marrow GLP, similar to OECD 489	Negative in liver Statistically significant increased of mean tail intensity values in bone marrow at 5 and 10 mg/kg Toxicity: some clinical signs including rapid and/or gasping respiration, staggering, lethargy, dark eyes in some animals at the two highest doses. No effect on body weight. No clinical chemistry, macroscopic and microscopic findings.	Reliability not evaluable considering the uncertainties linked to PEG 400 experimental result **Test material (EC name): TMPTA** Purity = 80.2%	Unpublished study report 29 (2018) Kirkland (2018)

7.9.5.3. Human information

No relevant information available

7.9.5.4. Summary and discussion of mutagenicity

GENETIC TOXICITY *IN VITRO*

Gene mutation in bacteria:

Four studies are available to assess the induction of gene mutations by TMPTA in bacteria. Negative results were found with *S. typhimurium* (TA 100, TA 1537 and TA 98) or *E. Coli* (WP2 *uvrA*/pKM101) with and without metabolic activation. Weak positive response was reported for TA 1535 only in the presence of metabolic activation in two out of the 4 studies (with rat S9 and hamster S9, respectively). In the first test (Unpublished study report 24,

1989), the detected increase varied between a factor of 1.6 and 4.8 with no dose dependency. This effect may be a reflection of toxicity at the higher concentrations. In the second test (Cameron, 1991), the detected increase was about 2.5 fold and was also not dose-dependent. The biological relevance of this finding is questionable in the absence of a dose-response relationship.

Studies in mammalian cells *in vitro*:

- CHO cells

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

- Mouse lymphoma L5178Y cells

In the first study (Unpublished study report 25, 1979), dose-related increases of mutant frequencies were reported in the absence of metabolic activation. The positive results were found with relative total growth (RTG) > 10%. The size of colonies was not reported to discriminate gene mutation or chromosomal aberration. In the presence of S9 activation, positive response was observed in 1 of the 3 independent experiments (the others were either equivocal or negative), only at the highest concentration associated with severe cytotoxicity (RTG = 4.8%). In summary, no consistent response was reported among the 3 experiments making the conclusion difficult.

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

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

- Human lymphocytes

Statistically significant and concentration-related increases in the frequency of cells with structural chromosomal aberrations were noted in two independent experiments, with and without metabolic activation. The positive response occurred at lower concentrations without metabolic activation (Unpublished study report 27, 2005).

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

GENETIC TOXICITY *IN VIVO*

In vivo cytogenicity:

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

The first study (Unpublished study report 28, 2006) was performed according to OECD 474 but presents some limitations from the guideline: low number of animals analyzable per group (less than 5 animals due to mortality in some groups) and mainly the fact that there was no evidence of bone marrow exposure. Indeed, even if 2 deaths were reported at the highest dose in males (reason unknown), no systemic effect was found in females. PCE/NCE ratio was not altered and plasma levels of the test substance were not investigated. Furthermore, no kinetics data was available in mice to estimate the distribution profile of TMPTA after oral exposure. Therefore, from this study, the negative result is questionable since there is no adequate evidence of target tissue (bone marrow) exposure.

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

During the Substance Evaluation process, FR-MSCA initially proposed to ask in a draft decision a micronucleus assay *in vivo* (OECD 474) in mice to clarify the clastogenic concern and to perform an adequate risk assessment for carcinogenicity. After the SEv process including comments from registrants, ECHA and member states, the final ECHA decision on 6th July 2016 stated that a Comet assay *in vivo* (OECD 489) in mice analyzing bone marrow and liver was required.

The *in vivo* Comet assay was submitted in January 2018. In this study, CD-1 female mice (6/group) were exposed to TMPTA in PEG 400 by slow intravenous bolus injection directly into the femoral vein via a surgical cannula (Unpublished study report 29, 2018). Only females were tested in the main study considering that there was no sex-difference in a range-finding study. A first experiment was performed at 2.5, 5 and 10 mg/kg (based on an initial range-finding study showing clonic convulsion and twitching at 20 mg/kg bw) on two consecutive days. Liver and bone marrow were sampled at necropsy, 30 minutes after the last administration. No increase of DNA damage was reported either in the liver and the bone marrow. Given the heterogeneity of the formulations, it was not possible to demonstrate exactly what the animals had been administered. Therefore, the laboratory decided to perform a new experiment . The second experiment consists on the intravenous administration of TMPTA at 5, 10 or 20 mg/kg bw on two consecutive days. The doses were selected based on a second range-finding study showing clinical effects (mainly clonic convulsion and hunched posture) and body weight loss at 30 mg/kg bw. In this second experiment, there were no dose-related increases in % hedgehogs in the liver and bone marrow. In the liver, the mean tail intensity values for all treated groups were not significantly increased. According to the authors, the mean tail intensity values were significantly increased at 5 and 10 mg/kg bw in the bone marrow, but not at 20 mg/kg bw.

Text Table 4: TMPTA: Summary of Group Mean Data – Bone Marrow, Experiment 2

Group / Dose Level (mg/kg/day)	Total No. Cells Scored	Tail Intensity Mean	SEM	Back-Transformed Difference from Vehicle	P-value	Significance	Mean %Hedgehogs
6F / Vehicle (0)	900	0.18	0.03	-	-	-	0.39
7F / TMPTA (5)	900	0.67	0.23	2.95	0.0001	P≤0.001	0.35
8F /TMPTA (10)	900	0.29	0.03	2.05	0.0060	P≤0.01	0.07
9F /TMPTA (20)	900	0.25	0.05	1.11	0.5668	NS	0.13
10F / EMS (150)	450	9.43	0.23	78.57	<0.0001	P≤0.001	0.99
Dose Response (Groups 6, 7, 8 & 9): 0.5365 (NS)							

Some limitations should be noted on this study:

First, the choice of the solvent is rather unusual. Due to its viscous properties and its anti-inflammatory properties, PEG 400 appears not a suitable solvent. The viscous properties of PEG 400 is not favourable to intravenous injection. In this context, and considering the solubility of TMPTA in organic solvents, it is not clear why a more common solvent have not been used (e.g. CMC or corn oil). In the literature, PEG 400 is reported as well tolerated in different species and by several routes, including IV route (Pandey *et al*., 2017; Gad *et al*., 2016; Healing *et al*., 2015; Thackaberry *et al*., 2010). However, publications report anti-inflammatory / anti-oxidant properties of PEG 400 as well as some protective effects

when administered with other substances (Ackland *et al*., 2010; Juarez-Moreno *et al.,* 2015, Ma *et al.,* 2017; Hodoshima *et al.*, 2004; Klugman *et al.,* 1981). In particular, pegylation is used in pharmaceutical sector in order to improve the tolerability of medicine. Considering that, PEG 400 may reduce or affect the intrinsic toxicity of TMPTA. It can be hypothesized that PEG 400 may counteract the irritation and oxidative stress induced by TMPTA that may contributed to DNA damage. In this context, it cannot be ruled out that using PEG 400 may mask/decrease the reactivity of TMPTA. Therefore, e-MSCA would like to alert on the use of this solvent in (geno)toxicity studies. More research is needed to characterize possible interferences of this solvent in toxicological studies.

Secondly, according to OECD guideline 489, "*animals should be given daily treatments over a duration of 2 or more days (i.e. two or more treatments at approximately 24 hour intervals), and samples should be collected once at 2-6 h (or at the Tmax) after the last treatment*". In contrast, in the study, the samples were collected 30 min after the last treatment. This short interval may be justified by the IV administration and thus an expected immediate Tmax, but there is no adequate kinetics study to confirm the relevance of this time sampling.

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

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

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

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

In conclusion, e-MSCA considers that the Comet assay presents various biais for concluding on genotoxicity of TMPTA.

According to the SEv decision, the comet assay was required in order to clarify the carcinogenic mode of action of TMPTA. Since DNA damages are not observed in the liver which is the main target organ for carcinogenicity, it is judged not proportionate to ask for a new *in vivo* genotoxicity assay (such as Comet assay or micronucleus assay) even if limitations have been identified in the Comet assay which decrease its reliability. Therefore, no further action is considered justified in this SEv framework at this point of time. The e-MSCA has submitted in February 2019 a CLH report for TMPTA, including a proposal for carcinogenicity endpoint. Even if the available results are not considered to fullfill criteria to classify TMPTA as a germ cell mutagen agent, genotoxicity data will be included in this CLH report to be discussed at the RAC level.

7.9.6. Carcinogenicity

7.9.6.1. Carcinogenicity: dermal

The results of studies on carcinogenicity after dermal administration are summarised in the following table:

Table 15. Studies on carcinogenicity after dermal administration

Method	Results	Remarks	Reference
mouse (C3H/HeJ) male 50 mg (nominal, per mouse per application) Vehicle: paraffin oil Exposure: 80 weeks (Twice a week) A group of 50 C3H/HeJ male mice received topical application of 50 mg of TMPTA to shaved area of the back, twice weekly for 80 weeks. A group of solvent control and positive control (0.05% benzo(a)pyrene in mineral oil) were also maintained along with no-treatment control group in the study. Parameters evaluated included clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations.	Non-carcinogenic effects: ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst Neoplastic effects: no effects	3 (not reliable) Disregarded experimental result **Test material (EC name): TMPTA** Purity not stated	Unpublished study report 30 (1982)
mouse (Tg.AC hemizygous) male/female, 15/sex/group 0, 0.75, 1.5, 3, 6, 12 mg/kg (nominal conc.) Vehicle: acetone Exposure: 6 months (5 days per week) Application on the backs of male and female Tg.AC mice five times per week for 6 months. Animals painted with acetone alone served as the control groups. Tissues from 15 sites were examined for every animal.	NOAEL local = 1.5 mg/kg bw (hyperplasia and hyperkeratosis of the skin) NOAEL for carcinogenicity = 3 mg/kg bw (squamous cell papilloma and carcinoma of the skin)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): TMPTA** Purity = 80%	NTP (2005)

Method	Results	Remarks	Reference
F344/N rats and B6C3F1 mice male/female; 65/sex/group 0.3, 1.0, 3.0 mg/kg (nominal conc.) Vehicle: acetone Exposure: 104 to 105 weeks (rats); 105 to 106 weeks (mice) – dermal application (5 times per week) Interim evaluations performed after 2, 13 and 52 weeks equivalent or similar to OECD Guideline 451	NOAEL local < 0.3 mg/kg bw for female rats. NOAEL local = 0.3 mg/kg bw for mice and male rats. NOAEL non-neoplastic systemic effect = 1 mg/kg bw/day (hyperplasia in the adrenal medulla in male mice) NOAEL non-neoplastic systemic effect = 3 mg/kg bw/day (no effect in rats and female mice) NOAEL carcinogenicity = 0.3 mg/kg bw/day (hepatocholangiocarcinoma in female mice) NOAEL carcinogenicity = 0.3 mg/kg bw/day (malignant mesothelioma in male rats) NOAEL carcinogenicity = 3 mg/kg bw/day for female rats and male mice (no effects)	2 (reliable with restrictions) key study experimental result **Test material (EC name): TMPTA** Purity > 78%	NTP (2012)

7.9.6.2. Carcinogenicity: other routes

No information available

7.9.6.3. Human information

No relevant information available

7.9.6.4. Summary and discussion of carcinogenicity

The NTP conducted an assay with TMPTA dermally applied to genetically modified strain of mouse (NTP, 2005). The Tg. AC hemizygous mice used contains an oncogene, v-Ha-ras transgene, so this model is genetically initiated and sensitive to dermal tumour promoters. Tg. AC hemizygous mice were administered 0, 0.75, 1.5, 3, 6 or 12 mg TMPTA/kg bw in

acetone 5 days per week for 28 weeks. A group of positive control received dermal applications of 12-O-tetradecanoylphorbol-13-acetate 3 days per week for 28 weeks. Survival and mean body weights of dose groups were similar to those of the vehicle controls. There were some effects on organ weights (liver, lung, heart and kidney) without corresponding histopathological findings. Increased incidences of minimal to moderate (mostly mild) hyperplasia of the epidermis (from 3 mg/kg bw), hyperkeratosis (from 3 mg/kg bw), and chronic active inflammation (from 6 mg/kg bw) also occurred at the site of application. A hematopoietic cell proliferation and myelodysplasia occurred in both male and female mice at the highest dose. These changes may be attributed to dermal inflammation. Mice had significantly increased incidences and multiplicity of squamous cell papillomas of the skin at the site of dermal application from 6 mg/kg bw (0%, 0%, 0%, 13%, 80%, 87% in males and 0%, 0%, 0%, 7%, 73%, 100% in females, for each dose, respectively). Squamous cell carcinomas occurred at the site of application in one female at 1.5, 6 and 12 mg/kg bw. These carcinomas appeared to arise within papilloma. Thus they were considered related to treatment and possibly the result of malignant conversion of papilloma. Increased incidences of forestomach squamous cell papilloma in female mice at 12 mg/kg bw (27%, 33%, 27%, 13%, 33%, 60% for each dose, respectively) may have been related to chemical administration since the incidence is higher than the common spontaneous rate (10-25% in hemizygous females (Mahler *et al.* 1998) and Eastin *et al.* (2001) cited in NTP (2005)).

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

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

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

Carcinogenic findings: No test-substance related increase in neoplastic lesions was found in male mice and female rats. In male mice, there was a significant increase in the incidence of alveolar/bronchiolar adenoma at 3 mg/kg bw; however, the alveolar/bronchiolar carcinoma decreased in this group. This was not considered treatment-related due to the absence of a significant positive trend, because the combined incidence of adenoma and carcinoma were not significantly increased and the incidences were within the historical control ranges.

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

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

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

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

The NOAEL for local effects is lower than 0.3 mg/kg bw in female rats and equals to 0.3 mg/kg bw in male rats and mice. The NOAEL for non-neoplastic systemic effect is set at 1 mg/kg bw/day in male mice based on the statistically increase in hyperplasia in the adrenal medulla. In rats and female mice, the NOAEL for non-neoplastic systemic effects is set at 3.0 mg/kg bw/day. The NOAEL for neoplastic effects is set at 0.3 mg/kg bw/day in female mice based on the increase in hepatocholangiocarcinoma. In male rats, the NOAEL for neoplastic effects is set at 1 mg/kg bw/day based on malignant mesothelioma. In female rats and male mice, the NOAEL for neoplastic effects is set at 3 mg/kg bw/day.

Finally, another study assessing the carcinogenic potential of TMPTA in mice is available (Unpublished study report 30, 1982). This study is inadequate and should be disregarded due to several limitations (only males, low frequency of application, unique concentration tested, lack of purity, poorly described). C3H/HeJ male mice received topical application of 50 mg of the test substance (5 % in white mineral oil) to shaved area of the back, twice weekly for 80 weeks. A group of solvent control and positive control (0.05% benzo(a)

pyrene in mineral oil) were also maintained along with no-treatment control group in the study. Parameters evaluated included clinical signs, mortality, body weight, gross pathology and histopathological (neoplastic and non-neoplastic) examinations. The gross observations made at necropsy were dark red lesions in lungs, liver tumors, kidney haemorrhages, enlarged spleen, skin ulcers, flaky skin, enlarged and grey lymph nodes, haemorrhages in stomach, grey or yellow spots in adrenals. Non-neoplastic histopathological lesions included ulcer, abscess, acanthosis, dysplasia, fibrosis, pigmentation, hyperkeratosis and retention cyst. No skin tumors were found in treated animals.

Conclusion:

TMPTA produced skin and forestomach neoplasms in Tg.AC hemizygous mouse model (NTP, 2005). Analysis of Tg.AC hemizygous mouse studies showed 77% accuracy in identifying known human carcinogens (Pritchard et al. 2003 cited in NTP 2012). Although this type of assay cannot be considered as a definitive proof of carcinogenicity, the findings suggest that TMPTA is likely to be carcinogenic in a 2-year bioassay.

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

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

According to CLP regulation, the increase in tumours reported in the NTP (2012) study justifies a classification of TMPTA as a carcinogenic substance (Carc. 2). In this context, France has submitted a CLH proposal for this endpoint.

Considering the database on genotoxicity of TMPTA, the carcinogenic mode of action is not expected to be related to a genotoxic mode of action.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

The results of studies on fertility are summarised in the following table:

Table 16. Studies on fertility

Method	Results	Remarks	Reference
Combined 28 day repeated dose toxicity study with the reproduction/developmental toxicity screening test Wistar Han rats (10/sex)	NOAEL local effect = 30 mg/kg bw/day NOAEL systemic effect, reproduction and development = 300 mg/kg bw/day	Reliability not evaluable considering the uncertainties linked to PEG 400	Unpublished study report 23 (2015)

Method	Results	Remarks	Reference
Vehicle: polyethylene glycol 400 Males exposed for 29 days (beginning 2 weeks prior mating). Females treated for 41-55 days (2 weeks prior mating up at least 4 days of lactation). 0, 30, 100, 300 mg/kg bw/day GLP, OECD 422 (1996)		Key study experimental result **Test material (EC name): TMPTA** Purity = 80.2%	

Table 17. Studies on developmental toxicity

Method	Results	Remarks	Reference
Female pregnant rat (Crl: COBS, CD(SD)BR) (22/group) oral: gavage 0 and 500 mg/kg bw (nominal conc.) Vehicle: corn oil Exposure: 10 d, from Day 6 to 15 of gestation, inclusive (Once daily) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal) < 500 mg/kg bw/day (nominal) (mortality, increased incidence of clinical signs and gross pathological effects) NOAEL (development): 500 mg/kg bw/day (nominal)	2 (reliable with restrictions) key study experimental result **Test material (EC name): TMPTA** Purity not stated	Unpublished study report 31 (1983)
Range-finding study Female New Zealand White rabbits (6-7/group) Oral gavage 0-50-100-200 mg/kg bw/day from gestation day 6 to gestation day 28. Addition of an extragroup exposed to 150 mg/kg bw/day after occurrence of possible treatment-related developmental findings in the definitive prenatal developmental study.	Decreased fetal body weight from 100 mg/kg bw/day Late resorption at 150 mg/kg bw/d Maternal toxicity at all doses but occurring in only one female at 50 and 100 mg/kg bw/day	2 (reliable with restrictions) Supporting study experimental result **Test material (EC name): TMPTA** Purity = 80.2%	Unpublished study report 22 (2015)

Method	Results	Remarks	Reference
Vehicle: 1% aqueous carboxymethyl cellulose with 0.1% Tween-80 Not GLP, no guideline			
Prenatal developmental toxicity study Female New Zealand White rabbits (23-24/group) Oral gavage 0-10-30-100 mg/kg bw/day from gestation day 6 to gestation day 28 (first part) 0-130 mg/kg bw/day from gestation day 6 to gestation day 28 (second part) Vehicle: 1% aqueous carboxymethyl cellulose with 0.1% Tween-80 GLP, OECD 414	NOAEL maternal = 130 mg/kg bw/day First part of the study: NOAEL development = 30 mg/kg bw/day Second part of the study: NOAEL development: < 130 mg/kg bw/day	2 (reliable with restrictions) Key study experimental result **Test material (EC name): TMPTA** Purity = 80.2%	Unpublished study report 23 (2015)

7.9.7.1. Effects on fertility

In a 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test, 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. Regarding general toxicity, local effect on forestomach were noted (see details in section 5.6.3). Based on this study, the NOAEL for parental generation is 30 mg/kg bw/day for local toxicity (forestomach irritation) and 300 mg/kg bw/day for systemic toxicity (no treatment-related effect). The reproductive and developmental NOAEL is 300 mg/kg bw/day (no treatment-related effect).

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).

7.9.7.2. Developmental toxicity

A prenatal developmental toxicity study is available in rats (Unpublished study report 31, 1983). The study was similar to OECD Guideline No. 414 (1981) with the following

limitations: only one tested dose, low number of examined dams and no specification of the tested material. TMPTA at 500 mg/kg bw was administered by oral gavage to pregnant rats from day 6 through day 15 of gestation. Compound-related maternal toxicity was observed and these effects consisted of decreased survival, increased incidence of clinical signs, and an increased incidence of gross pathology findings (mainly on the stomach). Isolated increased effect on food and water consumption values were observed. Since 4 females died and 3 females were not pregnant, only 15 litters can be examined, that is slightly lower than what is recommended in OECD guideline for an adequate evaluation of teratogenic toxicity. Compared to control group, a higher incidence of visceral variants was reported (8% vs 3% - mainly characterized as dilated renal pelves) but was not significant. In conclusion, the NOAEL is set < 500 mg/kg bw/day for maternal toxicity and at 500 mg/kg bw/day for development (no treatment-related effect).

A recent prenatal developmental study in rabbit is also available in response to the final decision of the compliance check performed by ECHA (Decision number: CCH-D-2114289316-41-01). A range-finding study and a definitive prenatal developmental toxicity study were submitted in 2018.

In the range-finding study, pregnant female rabbits were orally exposed (by gavage) to TMPTA in CMC-Tween 80 at doses of 0, 50, 100 or 200 mg/kg bw/day from gestation day 6 to 28 (Unpublished study report 22, 2015). Mortality of 3 females, clinical signs, decreased food and water consumption, loss of corrected body weight gain (GD6-28 = -11.8%) and reduced faeces production were observed at 200 mg/kg bw/day. At 100 and 50 mg/kg bw/day, one female of each group had decreased food consumption, reduced faeces production and body weight loss. There was no effect on the mean numbers of corporea lutea, implantation sites, viable or dead fetuses, early or late resorption or pre- or post-implantation loss. It should be noted that the number of pregnant females in the control group is very low (only 2/6 pregnant females). The reason of this low pregnancy rate is unknown according to the author. In foetuses, reduced body weight was noted in 2 litters at 200 mg/kg bw/day (-17%) and in 1 litter at 100 mg/kg bw/day. External examination did not reveal any malformation or variation in foetuses. However, the sensitivity of this result in the control and 200 mg/kg bw/day groups is rather low due to the low number of foetus obtained (17, 48, 44 and 25 foetuses for each groups, respectively).

Due to possible treatment-related developmental finding in the main study at 100 mg/kg bw/day (see below), an additional group at 150 mg/kg bw/day was further investigated in a range-finding study (without the addition of a further control group). At this dose, faeces abnormalities, loss of corrected body weight gain (GD6-28 = - 9.4%), reduced food and/or water consumption were noted. In addition, one female had an early delivery. An increase in late resorption (5% versus 0% in the control; exceeding historical control values) leading to a lower number of viable litters (92.2% versus 100% in the control) was noted. In foetuses, reduced body weight was noted in 2 litters. External examination did not reveal any malformation or variation in foetus.

In the main prenatal developmental toxicity study, pregnant female rabbits were orally exposed (by gavage) to TMPTA at doses of 0, 10, 30 or 100 mg/kg bw/day from gestation day 6 to 28 (Unpublished study report 23, 2015). In order to further investigate the possible treatment-related foetal findings reported at 100 mg/kg bw/day, two groups treated at 0 or 130 mg/kg bw/day were further investigated in a second part of the study. Because the two parts of the study are performed at 1 year interval, they should be considered as 2 independent experiments.

First part of the study (0, 10, 30, 100 mg/kg bw/day):

There were 5 unscheduled deaths, 1 in control group, 1 at 30 mg/kg bw/day and 3 at 100 mg/kg bw/day, none attributable to treatment. In dams, there was only a slight increase in reduced faeces production from 100 mg/kg bw/day. Increase in post-implantation loss was found at the medium dose of 30 mg/kg bw/day (8.5%) and outside the historical control data (0.6-7.9%) but was not dose-dependent. Increase in visceral malformations

(characterized by 2 small eye, 1 cataract, 2 liver oedema and 2 ascite) was noted at 100 mg/kg bw/day (1.3%, 0.6%, 1.7%, 4.3% in the control, low, medium and high dose group, respectively). By comparison, these malformations have been rarely - or not - found in the historical control database of the laboratory. Specifically, mean litter proportion for absent and/or small eyes in the high group was higher than the maximum historical control value (1.5 versus 0.5% per litter) and cataract was not seen previously among actual historical controls. Liver oedema and ascite were both observed in the 2 same foetuses. Of these related malformations, only ascites was seen previously in a single historical control foetus. The incidence of sternum with supernumerary ossification site (3.8%) was statistically significantly higher than control and upper than the historical control limit (1.6%) at 100 mg/kg bw/day.

Second part of the study (0 and 130 mg/kg bw/day):

There was a slightly lower food consumption and corrected body weight gain (-6.8% versus -6.2%; not statistically significant) at 130 mg/kg bw/day. A significant increase in pre-implantation loss was found at 130 mg/kg bw, which was interpreted as not related to treatment by the laboratory (treatment starting after implantation). In addition in this group, there was also a significant higher post-implantation loss (9.2% versus 3.6% per litter in control) and late resorption (5.4% versus 2% in control) that could be attributed to one female with 100% of late resorption. The litter size at 130 mg/kg bw/day was significantly lower than control (8.0 versus 9.4 foetuses/litter). In addition, a trend towards slightly lower mean fetal body weights was noted in fetuses without reaching statistical significance. Similar effects on visceral and skeletal development as reported in the first part of the study were not found at 130 mg/kg bw/day.

Overall, NOAEL for maternal toxicity should be set at 130 mg/kg bw/day. Regarding developmental toxicity, effects are reported at 100 mg/kg bw/day in the first part of the study (visceral malformations and skeletal anomaly) and at 130 mg/kg bw/day in the second part of the study (lethality). Even if effects reported at 100 mg/kg bw/day were not consistently observed at 130 mg/kg bw/day, their relevance cannot be totally excluded since only one dose was tested in the second part of the study. Thus, as a conservative approach, a NOAEL of 30 mg/kg bw/day is set for developmental toxicity.

Conclusion:

In conclusion, there is no treatment-effect on reproduction and development after TMPTA administration to rats by gavage in a Combined Repeated Dose Toxicity Study with a Reproduction/Developmental Toxicity Screening Test. However, the information provided in a OECD guideline 422, as such, does not allow to conclude on the hazardous properties of the registered substance with respect to the information requirement for Annex X, Section 8.7.2. Indeed, the OECD 422 study is designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing test Guidelines 443, as a standard requirement set in Annex X, Section 8.7.3. In addition, the available study followed the OECD guideline dated on 1996 and, thus, endocrine disruptor relevant endpoints were not included and developmental toxicity was only assessed until sacrifice on PND4.

In the absence of a full reproductive toxicity study with TMPTA, other toxicity studies can bring some information on the potential of TMPTA to affect reproduction.

- In a 90-day study performed by dermal route at doses up to 12 mg/kg bw TMTPA in rats and mice (NTP, 2005), no effect on reproductive organ, on sperm parameters (sperm count and mobility) and on oestrous cycle was reported, except a significant decrease in left testis weights without histopathological alteration in rats. Although the relative length of time spent in the oestrous stages differed significantly from

vehicle groups at 6 and 12 mg/kg bw in female mice, the differences were not considered biologically significant.
- In a dermal carcinogenicity study in mice (NTP, 2012), the incidence of uterine stromal polyps was statistically increased at the highest tested dose of 3 mg/kg bw/day. This increase also exceeded the historical control data.
- Potential developmental toxicity was identified in a prenatal developmental toxicity study in rabbit (Peter, 2016).

The eMSCA is of the opinion that the information described above may not fulfil the REACH standard information requirements for reproductive toxicity. The eMSCA therefore recommends ECHA to consider this substance in the prioritisation for CCH, to check if further data needs to be requested. No further action is required in the framework of this SEv.

7.9.8. Hazard assessment of physico-chemical properties

Explosivity

There are no chemical groups associated with explosive properties present in the molecule.
The following information is taken into account for any hazard / risk assessment:
Non explosive

Justification for classification or non-classification:
There are no chemical groups associated with explosive properties present in the molecule, thus according to REACh legislation, Annex VII, 7.11, column 2, the study does not need to be conducted.

Flammability

The available information on flammability is summarised in the following table:

Table 18. Information on flammability

Method	Results	Remarks	Reference
EU Method A.12 (Flammability (Contact with Water))	Evaluation of results: Non flammable Study results: Ignition on contact with water: no	2 (reliable with restriction) key study experimental result **Test material (EC name): 2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate**	Unpublished study report 32 (2010)

The flammability is determined according the test method A.12. "Flammability (contact with water) " as described in Directive 92/69/EEC

The following information is taken into account for any hazard / risk assessment:
Non flammable

Justification for classification or non-classification:
In the course of Water solubility study according to EU A.6 test method, it was realised that the registered substance can be mixed in water without development of gas. As no gas is developed when the registered substance gets in contact with water, the determination of the Flammability (EEC. A.12 (Contact with water)) is not applicable.

Flash point

The available information on flash point is summarised in the following table:

Table 19. Information on flash point

Method	Results	Remarks	Reference
closed cup EU Method A.9 (Flash-Point)	Flash point: 194.5 °C at 1 atm	2 (reliable with restriction) key study experimental result **Test material (EC name): 2-ethyl-2-[[(1-oxoallyl)oxy]methyl]-1,3-propanediyl diacrylate**	Unpublished study report 33 (2010)

The flash point of the substance was determined in accordance with the test EEC method A.9 "Flash Point".

The following information is taken into account for any hazard / risk assessment:
The substance shows a flash point at a temperature of 194.5 °C (1 atm). The substance is not flammable.

Justification for classification or non-classification:
The substance shows a flash point at a temperature of 194.5 °C (1 atm) according to the definition in the EEC, method A.9 (by means of Pensky-Martens apparatus, according to DIN EN ISO 2719). The substance is not flammable.

Oxidising potential

Based on the chemical structure, the substance cannot react exothermically with combustible materials.

The following information is taken into account for any hazard / risk assessment:
No oxidising properties

Justification for classification or non-classification:
Based on the chemical structure the substance is incapable of reacting exothermically with combustible materials. According to REACh legislation, Annex VII, 7.13, column 2, the study does not need to be conducted.

No further action is required for these endpoints in the framework of this SEv.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The registrants have derived DNELs for systemic long-term effects for workers (by dermal and inhalation routes) and general population (by dermal, inhalation and oral routes). The DNELs are derived from the OECD 422 study by oral route. No quantitative risk characterisation was performed for local effects considering TMPTA as a skin sensitizer and irritant.

No DNEL has been established by e-MSCA during substance evaluation. E-MSCA questions the relevance of using results of the OECD 422 study considering the methodological limitations described in the corresponding section of this document. In addition, e-MSCA identified a lower oral NOAEL from the prenatal developmental toxicity study. Finally, 2-year dermal studies are available and seem more relevant to be used as point of departure to characterize the risk following dermal exposure. However, e-MSCA considered that a more thorough risk characterization is not needed in this SEv framework based on the intended uses and the skin sensitisation and irritant properties of TMPTA (see arguments below).

Oral route:

Registrants have declared consumer uses as uses advised against. Therefore, no further risk characterization is needed for direct exposure. The risk to man via the environment has not be assessed in this SEv framework.

Dermal route:

TMPTA is classified as irritant and sensitizing to the skin according to CLP regulation. Therefore, appropriate personal protective equipments, such as gloves and protective clothing, should be worn to avoid skin contact. In the repeated-dose toxicity studies by dermal routes (NTP, 2005 and 2012), systemic effects occurred at doses higher than those associated with local irritating effects. In this context, it is expected that wearing appropriate personal protective equipements is sufficient to avoid the occurrence of both local and systemic effects.

Registrants have declared consumer uses as uses advised against. Therefore, no further risk characterization is needed for direct exposure.

Inhalation route:

Following the decision under SEv, registrants have deleted a spray scenario (PROC 7) from their CSR. In addition, considering the low vapour pressure, inhalative exposure is considered rather low. Finally, since TMPTA is classified as a skin sensitizer, breathing dust/fume/gas/mist/vapours/spray should be avoided according to CLP regulation. In this context, no specific or further quantitative risk assessment is needed for inhalative exposure.

Registrants have declared consumer uses as uses advised against. Therefore, no further risk characterization is needed for direct exposure.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

TMPTA is not acutely toxic.

Regarding local effects, TMPTA has irritating and skin sensitising properties and is accordingly classified in the CLP regulation.

After repeated-dose toxicity by dermal route, carcinogenic effects are reported in female mice (stromal polyps, hepatoblastoma and hepatocholangiocarcinoma) and in male rats (malignant mesothelioma). Based on these results, the current EU harmonized classification for TMPTA should be updated by adding Carc. 2.

In a combined 28 day repeated dose toxicity study with the reproduction/developmental toxicity screening test only local effects are found.

Concerning genotoxicity, *in vitro* dataset show clastogenic effect of TMPTA. The available *in vivo* micronucleus studies are not considered reliable to conclude on this endpoint. In this context, an *in vivo* Comet assay was required during the SEv process. TMPTA does not induce DNA damage in the liver but the mean tail intensity was increased in the bone marrow at low and mid doses (without dose-response relationship). Genotoxicity data will be included in a CLH report, at the light of the carcinogenic effects, to be discussed at the RAC level.

Regarding toxicity to fertility, only a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test is available. No reproductive or developmental effects were found. E-MSCA recommends ECHA to consider to prioritise this substance for CCH for this endpoint to check if further data are needed with regard to Reach requirements. In a prenatal developmental toxicity study in rabbits, some developmental effects (including visceral malformations, skeletal anomalies and lethality) were noted. However, an harmonized classification as a reproductive toxicant is not considered appropriate at this stage.

7.10. Assessment of endocrine disrupting (ED) properties

No adequate data is available to assess endocrine disrupting properties of TMPTA for environment or human health. However, no specific concern has been identified based on available data.

At the time being, no further action is required for these endpoints in the framework of this SEv. Some concern may be raised in a further EOGRTS if requested by ECHA in a CCH.

7.11. PBT and VPVB assessment

The substance is readily biodegradable and its estimated bioaccumulation potential is low (BCF = 4.26). Based on the assessment described in the subsections above, TMPTA is not a PBT / vPvB substance in accordance with Annex XIII to Regulation (EC) No 1907/2006.

7.12. Exposure assessment

7.12.1. Human health

7.12.1.1. Worker

Not specifically assessed during the evaluation of the substance.

TMPTA is used at industrial sites (formulation of preparations, application of all coatings and inks in dry process, use in polymer industry) and by professional workers (indoor printing with ink cartridges in dry process).

The following uses advised against are declared by the registrants : perfumes, fragrances, cosmetics, personal care products, dental and fingernail application.

According to the final decision (06 July 2016), the registrant(s) shall submit "Detailed description and justifications for each contributing scenarios and revision of spraying scenarios with appropriate models". In 2018, the registrants updated their CSR and deleted the spray scenario. Therefore, the initial concern related to wide dispersive uses and high RCR has been clarified.

7.12.1.2. Consumer

Used advised against. No concern identified.

7.12.2. Environment

Information on the process, technical on-site conditions, measures to reduce or limit emissions are adequately described to ensure that environmental releases are adequately estimated in sewage treatment plant (STEP), surface water, sediment and soil compartments.

7.12.3. Combined exposure assessment

Not assessed during the evaluation of the substance.

7.13. Risk characterisation

Human Health

Not specifically assessed during the evaluation of the substance.

Environment

Risks for environment are adequately controlled for all environmental compartments. No risk for secondary poisoning is detected.

7.14. References

Ackland GL, Gutierrez Del Arroyo A, Yao ST, Stephens RC, Dyson A, Klein NJ, Singer M, Gourine AV (2010).Low-molecular-weight polyethylene glycol improves survival in experimental sepsis. Crit Care Med. 2010 Feb;38(2):629-36.

Björkner B, Dahlquist I, Fregert S (1980). Allergic contact dermatitis from acrylates in ultraviolet curing inks. Contact Dermatitis. 1980 Oct;6(6):405-9.

Cameron TP, Rogers-Back AM, Lawlor TE, Harbell JW, Seifried HE, Dunkel VC (1991). Genotoxicity of Multifunctional Acrylates in the Salmonella/Mammalian-Microsome Assay and Mouse Lymphoma TK+/- Assay. Environmental and Molecular Mutagenesis 17:264-271.

Christoffers WA, Coenraads PJ, Schuttelaar MLA (2013). Two decades of occupational (meth)acrylate patch test results and focus on isobornyl acrylate. Contact Dermatitis, 69, 86-92

Dahlquist I, Fregert S, Trulson L (1983). Contact allergy to trimethylolpropane triacrylate (TMPTA) in an aziridine plastic hardener. Contact dermatitis 1983: 9: 122-124.

Davis B (2012). Endometrial Stromal Polyps in Rodents: Biology, Etiology, and Relevance to Disease in Women. Toxicol Pathol 2012 40: 419

Dearfield KL (1989). Analysis of the genotoxicity of nine acrylate/methacrylate compounds in L5178Y mouse lymphoma cells. Mutagenesis Vol. 4, No. 5, pp.381-393.

EC (2003). European Commission Technical Guidance Document (TGD) on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II - Chapter 3 - Environmental Risk Assessment. https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf

ECHA (2008). Guidance on information requirements and chemical safety assessment - Chapter R.10: Characterisation of dose [concentration]-response for environment. May 2008. https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-a503-4ab7-9036-d866b8ddce69

ECHA (2010). Guidance on information requirements and Chemical Safety Assessment. Chapter R.16: Environmental exposure assessment. Version 3.0. February 2016. https://echa.europa.eu/documents/10162/13632/information_requirements_r16_en.pdf/b9f0f406-ff5f-4315-908e-e5f83115d6af

EFSA (2017). Guidance on dermal absorption. 24 May 2017. doi: 10.2903/j.efsa.2017.4873

Emmett EA, Kominsky JR (1977). Allergic Contact Dermatitis from Ultraviolet Cured Inks. Journal of Occupational Medicine/Vol. 19, No. 2/Feb 1977.

Gad SC, Spainhour CB, Shoemake C, Stackhouse Pallman DR, Stricker-Krongrad A, Downing PA, Seals RE, Eagle LA, Polhamus K, and Daly J (2016). International Journal of Toxicology 2016, Vol. 35(2) 95-178

Greaves P (2007). Histopathology of preclinical toxicity studies. 3rd Edition

Hayes BB, Meade BJ (1999). Contact sensitivity to selected acrylate compounds in B6C3F1 mice: relative potency, cross reactivity and comparison of test methods. Drug and chemical toxicology, 22(3), 491-506.

Healing G, Sulemann T, Cotton P, Harris J, Hargreaves A, Finney R, Kirk S, Schramm C, Garner C, Pivette P and Burdett L (2016). Safety data on 19 vehicles for use in 1 month oral rodent pre-clinical studies: administration of hydroxypropyl-ß-cyclodextrin causes renal toxicity. J. Appl. Toxicol. 2016; 36: 140–150

Hodoshima N, Nakano Y, Izumi M, Mitomi N, Nakamura Y, Aoki M, Gyobu A, Shibasaki S, Kurosawa T (2004). Protective effect of inactive ingredient against nephrotoxicity of vancomycin hydrochloride in rats. Drug Metab. Pharmacokin. 19(1): 68-75

IARC (2018). Carcinogenicity of isobutyl nitrite, β-picoline, and some acrylates. The Lancet. 9(8):1020-1022

Juarez-Moreno K, Ayala M, Vazquez-Duhalt R. (2015) Antioxidant Capacity of Poly(Ethylene Glycol) (PEG) as Protection Mechanism Against Hydrogen Peroxide Inactivation of Peroxidases. Appl Biochem Biotechnol 177:1364–1373

Kanerva L, Tarvainen K, Jolanki R, Henriks-Eckerman ML, Estlander T (1998). Short communication. Airborne occupational allergic contact dermatitis due to trimethylolpropane triacrylate (TMPTA) used in the manufacture of printed circuit boards. Contact Dermatitis, 1998, 38, 292-294.

Kirkland D, Fowler P (2018). A review of the genotoxicity of trimethylolpropane triacrylate (TMPTA). Mutat Res Gen Tox En 828 (2018) 36–45

Klugmann FB, Decorti G, Mallardi F, Klugmann S, Baldini L (1984). Effect of polyethylene glycol 400 on adriamycin toxicity in mice. Eur J Cancer Clin Oncol. 1984 Mar;20(3):405-10.

Ma BL, Yang Y, Dai Y, Li Q, Lin G and Ma YM (2017). Polyethylene glycol 400 (PEG400) affects the systemic exposure of oral drugs based on multiple mechanisms: taking berberine as an example. RSC Adv., 2017, 7, 2435–2442

Mancuso G and Berdondini RM (2008). Occupational conjunctivitis as the sole manifestation of airborne contact allergy to trimethylolpropane triacrylate contained in a UV-cured paint. Contact Dermatitis 2008: 59: 372-373.

Maronpot RR, Zeiger E, McConnell EE, Kolenda-Roberts H, Wall H, and Friedman MA (2009). Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. Critical Reviews in Toxicology, 2009; 39(6): 512–537

Moore MM, Harrington-Brock K, Doerr CL, Dearfield KL (1989). Differential mutant quantitation at the mouse lymphoma tk and CHO hgprt loci. Mutagenesis vol. 4 no 5 pp.394-403.

Nethercott, J. R., Jakubovic, H. R., Pilger, C., and Smith, J. W. (1983). Allergic contact dermatitis due to urethane acrylate in ultraviolet cured inks. British Journal of Industrial Medicine 1983; 40:241-250. Study number: CLIT 17280.

Nethercott JR (1978). Skin problems associated with multifunctional acrylic monomers in ultraviolet curing inks. British Journal of dermatology (1978) 98, 541

NTP (2005). NTP report on the toxicology studies of trimethylolpropane triacrylate (technical grade) (CAS No. 15625-89-5) in F344/N rats, B6C3F1 mice and genetically modified (FVB Tg. Ac hemizygous) mice (dermal studies).

NTP (2012). Toxicology and carcinogenesis studies of trimethylolpropane triacrylate (technical grade) in F344/N rats and B6C3F1/N mice.

Pandey SK, Goyal VK, Nalge P, Are P, Vincent S, Nirogi R (2017). Assessment of toxicity and tolerability of a combination vehicle; 5% Pharmasolve, 45% Propylene glycol and 50% Polyethylene glycol 400 in rats following repeated intravenous administration. Regulatory Toxicology and Pharmacology 91 (2017) 103–108

Sanchez-Garcia S, Fernandez-Nieto M, Sastre J (2009). Asthma induced by a thermal printer. N Engl J Med. 2009 May 28;360(22):2375-6.

Thackaberry EA, Kopytek S, Sherratt P, Trouba K,‡ and McIntyre B (2010). Comprehensive Investigation of Hydroxypropyl Methylcellulose, Propylene Glycol, Polysorbate 80, and Hydroxypropyl-Beta-Cyclodextrin for use in General Toxicology Studies. Toxicological Sciences 117(2), 485–492 (2010)

Unpublished study report 1 (2010). 'Confidential Substance name' Ready Biodegradability - Evaluation of the Aerobic Biodegradability in an Aqueous Medium: OECD 301 B: CO2 Evolution Test.

Unpublished study report 2 (2016). Trimethylolpropane triacrylate (CAS 15625-89-5) – Fish, acute toxicity test.

Unpublished study report 3. (1988). Report on the study of acute toxicity. Test substance: Trimethylolpropantriacrylat, Species: Golden orfe (Leuciscus idus).

Unpublished study report 4 (1991). Bestimmung der akuten Wirkung von Trimethylolpropantriacrylat auf die Schwimmfähigkeit des Wasserflohs Daphnia magna (Straus).

Unpublished study report 5 (1988). Determination of the acute toxicity of Trimethylolpropantriacrylat to the waterflea Daphnia magna Straus.

Unpublished study report 6 (1989). Inhibition of the algae cell multiplication according to DIN 38412 L9.

Unpublished study report 7 (1972). Toxicity studies acrylate monomers.

Unpublished study report 8 (1978). Acute oral toxicity study of "TMPTA" in the rat.

Unpublished study report 9 (1976). Acute vapour inhalation toxicity study in rats.

Unpublished study report 10 (2015). *In vitro* percutaneous absorption of neat Trimethylolpropane triacrylate (TMPTA) through human skin.

Unpublished study report 11 (1981). Prüfung der akuten dermalen Toxizität von "TMPTA" an der Ratte.

Unpublished report 12 (1980). Prüfung der akuten Inhalationsgefahr (akutes Inhalationsrisiko) von "TMPTA" an Sprague-Dawley-Ratten.

Unpublished study report 13 (1978). Primary skin and eye irritation tests with trimethylolpropanetriacrylate I in albino rabbits.

Unpublished study report 14 (1978). Primary skin and eye irritation tests with trimethylolpropanetriacrylate II in albino rabbits.

Unpublished study report 15 (1977). Primary skin irritation tests with 8 multifunctional acrylate monomers in albino rabbits.

Unpublished study report 16 (1990). Skin irritation to the rabbit of 'Confidential Substance name'.

Unpublished study report 17 (2018). Evaluation of the Eye Hazard Potential of TMPTA using the Bovine Corneal Opacity and Permeability Test (BCOP Test).

Unpublished study report 18 (1974). Skin sensitization test with trimethylolpropane triacrylate in albino guinea pigs.

Unpublished study report 19 (2015). 14-day dose range finding study for a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of TMPTA in rats by oral gavage.

Unpublished study report 20 (2015). Combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of TMPTA in rats by oral gavage.

Unpublished study report 21 (1979). A two/four week dermal toxicity study in rabbits.

Unpublished study report 22 (2015). Dose range finding study in pregnant rabbits for a prenatal developmental toxicity study of TMPTA in rabbits by oral gavage.

Unpublished study report 23 (2015). Prenatal developmental toxicity study of TMPTA in rabbits by oral gavage.

Unpublished study report 24 (1989). Trimethylolpropantriacrylat (ZST Test Substance No.: 88/998) in the AMES TEST.

Unpublished study report 25 (1976). Evaluation of TMPTA, PETA and PEGDA in the Salmonella/microsome mutagenicity test.

Unpublished study report 26 (1979). Mutagenicity evaluation of trimethylolpropane triacrylate in the mouse lymphoma forward mutation assay.

Unpublished study report 27 (2005). In Vitro Mammalian Chromosome Aberration Test in Cultured Human Lymphocytes.

Unpublished study report 28 (2006). Bone marrow micronucleus test by oral route in mice.

Unpublished study report 29 (2018). TMPTA : mouse alkaline comet assay.

Unpublished study report 30 (1982). Chronic mouse dermal toxicity study.

Unpublished study report 31 (1983). Teratology screen in rats.

Unpublished study report 32 (2010). Determination of the Flammability (EEC A.12.) of the test item 'Confidential Substance name'.

Unpublished study report 33 (2010). Determination of the Flash Point (EEC A.9.) of the test item 'Confidential Substance name'.

7.15. Abbreviations

ANSES et du travail	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement (French Agency for Food, Environmental and Occupational Health & Safety)
BCF	Bioconcentration Factor
BW	Body weight
CAS	Chemical abstracts service
CCH	Compliance check
CCPP	Occupational disease consultation centres
CHO	Chinese hamster ovary
CLH	Harmonized classification
CLP	Classification, labelling and packaging (Regulation (EC) No 1272/2008)
CMC	Critical micelle concentration
CMC	Carboxymethyl cellulose
CoRAP	Community Rolling Action Plan
CSR	Chemical safety report
CYP	Cytochrome

DMEL	Derived minimal effect level
DNEL	Derived no effect level
DT50	Disappearance Time 50 is the time within which the concentration of the test substance is reduced by 50%
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
eMSCA	Evaluating Member State Competent Authority
EOGRTS	Extended one-generation reproductive toxicity study
ERC	Environmental release category
FEV_1	Forced expiratory volume in one second
FE_{NO}	Fraction of nitric oxide in exhaled air
FR	France
GD	Gestational day
GLP	Good laboratory practice
HPLC	High-performance liquid chromatography
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
IARC	International agency for research on cancer
IUCLID	International Uniform Chemical Information Database
IV	Intravenous
IVIS	*In vitro* irritancy score
LD_{50}	Median lethal dose. The dose causing 50 % lethality
MEST	Mouse ear swelling test
MSC	Member State Committee
MSCA	Member State Competent Authority
NCE	Normochromatic erythrocytes
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOEL	No observed effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocytes
PBT	Persistent, Bioaccumulative, Toxic

PEG 400	Polyethylene glycol 400
PETA	Pentaerythritol triacrylate
PNEC	Predictive No Effect Concentration
PROC	Process category
QSAR	Quantitative structure–activity relationship
RAC	Risk Assessment Committee
RIVM	Het Rijksinstituut voor Volksgezondheid en Milieu
RNV3P	National occupational disease surveillance and prevention network
RTG	Relative total growth
SEv	Substance Evaluation
STOT SE	Specific target organ toxicity after single exposure
SVHC	Substance of very high concern
$ThCO_2$	Theoretical carbon dioxide (mg) is the quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test compound when fully mineralized; also expressed as mg carbon dioxide volved per mg test compound
TMPTA	Trimethylolpropane triacrylate
TMPTMA	Trimethylol propane trimethacrylate
vPvB	Very Persistent and very Bioaccumulative

A confidential Annex was removed.