Ministry of Environment of Denmark
Environmental Protection Agency

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2,3-epoxypropyl o-tolyl ether

EC No 218-645-3

CAS RN 2210-79-9

Evaluating Member State(s): Denmark

Dated: 31 January 2022

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2016

Before concluding the substance evaluation a Decision to request further information was issued on: 3 January 2018.

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 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

The Substance, 2,3-epoxypropyl o-tolyl ether (EPOTE; EC No 218-645-3, CAS RN 2210-79-9), was originally selected for substance evaluation to clarify concerns about:

- Mutagenicity

During the evaluation additional concerns were identified:

- Skin sensitisation
- Carcinogenicity
- Exposure of consumers and workers

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Not applicable

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.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
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	

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 Substance EPOTE is currently classified as Skin Irrit. 2, Skin Sens. 1, Muta. 2 and Aquatic Chronic 2.

- Skin sensitisation

The available Guinea pig maximization studies on the Substance indicated a high skin sensitising potency but were not suited for sub-categorization 1A or 1B. The requested LLNA study showed a dose-response relationship with an EC3 of 1.3%. According to CLP, skin sensitizers with EC3 values ≤ 2% are considered strong sensitizers (Unpublished report, 2019a).
Thus, the LLNA points to classification of the Substance EPOTE as a strong sensitiser category 1A. As most notifications for classification concerning skin sensitisation, including

that of the registrants do not include classification as Skin Sens. 1A, the eMSCA considers that harmonisation of the classification of EPOTE for skin sensitisation is relevant.

- Mutagenicity

The Transgenic Rodent Gene Mutation Assay (TGR, OECD TG 488) in germ cells, requested to clarify whether a Muta 1 classification could be warranted, was negative (Unpublished report, 2019b). Thus, the available data on mutagenicity supports the current classification as Muta. 2.

The eMSCA plans to submit a registry of intension for a classification proposal for Skin Sens cat 1A in March 2022. Submission of the CLH dossier is planned for January 2023.

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

Not applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

Not applicable

5.2. Other actions

Not applicable

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**
Initiate CLP Annex VI dossier (Proposal for Skin Sens. 1A)	Tentative January 2023	DK

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance EPOTE (2,3-epoxypropyl o-tolyl ether, EC No 218-645-3, CAS RN 2210-79-9) was originally selected for substance evaluation to clarify concerns about:

- Mutagenicity

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

- Skin sensitisation
- Carcinogenicity
- Exposure of consumers and workers

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Mutagenicity	**Concern refuted.** No further action needed under SEv
Skin Sensitisation	**Concern confirmed.** Classification proposal is planned
Carcinogenicity	**Concern unresolved.** Compilation of justification for possible future action is planned - please see section 7.9.6
Exposure of consumers and workers	**Concern unresolved.** Ambiguous but currently no further action – please see section 7.12

7.2. Procedure

The registered Substance 2,3-epoxypropyl o-tolyl ether (EPOTE; EC No 218-645-3, CAS RN 2210-79-9) was included in the Community rolling action plan (CoRAP) for substance evaluation in 2016. The competent authority of Denmark (hereafter called the evaluating MSCA (eMSCA)) was appointed to carry out the evaluation in accordance with Article 45(4) of REACH based on the information in the REACH registration(s) and other relevant and available information.

During the evaluation, the eMSCA identified additional concerns regarding skin sensitisation, carcinogenicity and exposure of consumers and workers.

The evaluating MSCA considered that further information was required to clarify the above-mentioned concerns. Therefore, a draft decision (DD) under Article 46(1) of REACH was prepared and submitted to ECHA on 16 March 2017.

ECHA notified the registrant of the draft decision (DD) and invited for comments which were received on 2 June 2017. The eMSCA took the comments into account and amended the DD, which was then included in the Member State Committee decision seeking stage.

The Member State Committee reached a unanimous agreement on the revised DD in its MSC-57 written procedure and ECHA took the decision according to Article 51(6) of REACH. The decision making followed the procedure of Articles 50 and 52 of REACH.

The final decision, published on ECHAs website 3 January 2018[2], requested the following: (i) A transgenic rodent somatic germ cell assay (OECD TG 488) in mice to clarify the mutagenicity concern, (ii) a Local Lymph Node Assay (OECD TG 429) to clarify the concern for skin sensitisation, and (iii) adaptations/specifications in the CSR regarding exposure and calculations of Risk Characterisation Ratios (RCR's) for consumers and workers.

In September 2020, the registrant updated the registration dossier to ECHA including the study report for the TGR-study, the LLNA study and an updated version of the CSR. The eMSCA launched a follow-up evaluation on the substance on 27 October 2020.

The Follow-up evaluation was concluded with the present conclusion report. No further information is requested under this substance evaluation.

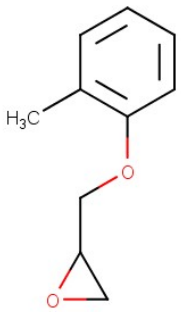
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	2,3-epoxypropyl o-tolyl ether
EC number:	218-645-3
CAS number:	2210-79-9
Index number in Annex VI of the CLP Regulation:	603-056-00-X
Molecular formula:	C10H12O2
Molecular weight range:	164.201
Synonyms:	Oxirane, (2-methylphenoxy)methyl - o-Cresyl glycidyl ether Glycidyl o-tolyl ether

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

Structural formula:



[2] https://echa.europa.eu/documents/10162/be672c19-e94a-50f5-d7f0-1cf581adaf5a

7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Colourless liquid
Vapour pressure	0.514 Pascal at 20°C and 0.822 Pascal at 25°C(OECD TG 104)
Water solubility	Appr. 0.84 g/L , moderately soluble (100-1000 mg/L) (OECD TG 107)
Partition coefficient n-octanol/water (Log Kow)	2.50 +/- 0.062. (OECD TG 107)
Flammability	Data waived
Flash point	123.4 +/- 2.14 °C at 30.0 mmHg. (EU test method A9)
Explosive properties	Data waived
Oxidising properties	Data waived
Granulometry	Data waived
Stability in organic solvents and identity of relevant degradation products	Data waived
Dissociation constant	Data waived
Freezing point/ melting point	Freezing point < -69°C (OECD TG 102)
Boiling point	260 +/- 0.29 °C (OECD TG 103)
Density	1.09 (OECD TG 109)
Viscosity	9.64 cSt +/- 0.03 cSt at 20 °C and 4.72 cSt +/- 0.01 cSt at 40 °C. (OECD TG 114)

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	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.1.

7.5.2. Overview of uses

Table 7

USES	
	Use(s)
Uses as intermediate	Environment release categories (ERCs) ERC 0: Other (mERC I.1, mERC I.2 (mERC: company derived spERC's) Process categories (PROCs) PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment condition PROC 4: Chemical production where opportunity for exposure arises PROC 5: Mixing or blending in batch processes PROC 6: Calendering operations PROC 7: Industrial spraying PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 11: Nonindustrial spraying PROC 13: Treatment of articles by dipping and pouring PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 15: Use as laboratory reagent PROC 16: Use of fuels PROC 19: Manual activities involving hand contact PROC 20: Use of functional fluids in small devices Sector of end use: SU 1: Agriculture, forestry and fishing SU 2a: Mining (without offshore industries) SU 2b: Offshore industries SU 5: Manufacture of textiles, leather, fur SU 6a: Manufacture of wood and wood products SU 6b: Manufacture of pulp, paper and paper products SU 7: Printing and reproduction of recorded media SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 10: Formulation (mixing) of preparations and/or re-packaging (excluding alloys) SU 11: Manufacture of rubber products SU 12: Manufacture of plastics products, including compounding and conversion SU 13: Manufacture of other non-metallic mineral products e.g., plasters, cement SU 15: Manufacture of fabricated metal products, except machinery and equipment SU 16: Manufacture of computer, electronic and optical products, electrical equipment SU 17: General manufacturing, e.g., machinery, equipment, vehicles, other transport equipment SU 18: Manufacture of furniture

	SU 19: Building and construction work SU 23: Electricity, steam, gas, water supply and sewage treatment SU 24: Scientific research and development
Formulation	Environment release categories (ERCs) ERC1: Manufacture of the substance Process categories (PROCs) PROC3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions
Uses at industrial sites	Environment release categories (ERCs) ERC0: Other (mERC l.1, mERC l.2 (mERC: company derived spERC's) Process categories (PROCs) PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment condition PROC 4: Chemical production where opportunity for exposure arises PROC 5: Mixing or blending in batch processes PROC 6: Calendering operations PROC 7: Industrial spraying PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 13: Treatment of articles by dipping and pouring PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 15: Use as laboratory reagent PROC 16: Use of fuels PROC 19: Manual activities involving hand contact Sector of end use: SU 1: Agriculture, forestry and fishing SU 2a: Mining (without offshore industries) SU 2b: Offshore industries SU 5: Manufacture of textiles, leather, fur SU 6a: Manufacture of wood and wood products SU 6b: Manufacture of pulp, paper and paper products SU 7: Printing and reproduction of recorded media SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 10: Formulation (mixing) of preparations and/or re-packaging (excluding alloys) SU 11: Manufacture of rubber products SU 12: Manufacture of plastics products, including compounding and conversion SU 13: Manufacture of other non-metallic mineral products e.g., plasters, cement SU 15: Manufacture of fabricated metal products, except machinery and equipment SU 16: Manufacture of computer, electronic and optical products, electrical equipment SU 17: General manufacturing, e.g., machinery, equipment, vehicles, other transport equipment SU 18: Manufacture of furniture

	SU 19: Building and construction work SU 23: Electricity, steam, gas, water supply and sewage treatment SU 24: Scientific research and development
Uses by professional workers	Environment release categories (ERCs) ERC 0: Other (mERC I.1, mERC I.2 (mERC: company derived spERC's) Process categories (PROCs) PROC 5: Mixing or blending in batch processes PROC 6: Calendering operations PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing) PROC 11: Nonindustrial spraying PROC 13: Treatment of articles by dipping and pouring PROC 14: Tabletting, compression, extrusion, pelletisation, granulation PROC 15: Use as laboratory reagent PROC 16: Use of fuels PROC 19: Manual activities involving hand contact PROC 20: Use of functional fluids in small devices Sector of end use: SU 1: Agriculture, forestry and fishing SU 5: Manufacture of textiles, leather, fur SU 6a: Manufacture of wood and wood products SU 6b: Manufacture of pulp, paper, and paper products SU 7: Printing and reproduction of recorded media SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 10: Formulation [mixing] of preparations and/or re-packaging (excluding alloys) SU 11: Manufacture of rubber products SU 12: Manufacture of plastics products, including compounding and conversion SU 13: Manufacture of other non-metallic mineral products, e.g., plasters, cement SU 15: Manufacture of fabricated metal products, except machinery and equipment SU 16: Manufacture of computer, electronic and optical products, electrical equipment SU 17: General manufacturing, e.g., machinery, equipment, vehicles, other transport equipment SU 18: Manufacture of furniture SU 19: Building and construction work SU 24: Scientific research and development
Consumer Uses	The substance is marketed and used in industrial and professional uses only according to the registrants and there is no description of consumer exposure in the substance dossier. However, according to the Nordic product register (SPIN database: http://www.spin2000.net/spinmyphp/) this substance is used in products with consumer exposure.
Article service life	Not evaluated by the eMSCA. Not relevant according to the registrant.

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)		
603-056-00-X	2,3-epoxypropyl o-tolyl ether	218-645-3	2210-79-9	Skin Irrit. 2 Skin Sens. 1 Muta. 2 Aquatic Chronic 2	H315 H317 H341 H411		Note C

7.6.2. Self-classification

- In the registration(s):

Aquatic Chronic 2 (H411: Toxic to aquatic life with long lasting effects)
Skin Irrit. 2 (H315 : Causes skin irritation)
Skin Sens. 1A (H317: May cause an allergic skin reaction)

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects)
Acute Tox. 3 (H331: Toxic if inhaled)
Acute Tox. 4 (H302: Harmful if swallowed; H312: Harmful in contact with skin)
Eye Irrit. 2 (H319: Causes serious eye irritation)
Muta 2 (H341: Suspected of causing genetic defects)
Skin Corr. 1B (H314: Causes severe skin burns and eye damage)
STOT SE 3 (H335: May cause respiratory irritation)
Skin Sens. 1 (H317: May cause an allergic skin reaction)

7.7. Environmental fate properties

Not evaluated by the eMSCA

7.8. Environmental hazard assessment

Not evaluated by the eMSCA

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Not evaluated by the eMSCA

7.9.2. Acute toxicity and Corrosion / Irritation

Not evaluated by the eMSCA

7.9.3. Sensitisation

The Substance EPOTE is categorised as having a high sensitizing potency, which has been shown experimentally as well as by human data. The substance has a harmonised classification as a skin sensitiser (Skin Sens 1) according to CLP. It is also classified as irritant to the skin (Skin irrit 2). The endpoint of skin sensitisation was selected for substance evaluation of EPOTE to assess the skin sensitising potency of a substance.

7.9.3.1. Animal data

Guinea pig maximisation test (1989)

A Guinea pig maximisation test was performed in 1989 according to OECD TG 406 (version 1981) with GLP compliance. However, the test substance was only identified by trade name (not chemical name or CAS Number), but assumed to be EPOTE, as the study was included in the registration. No information regarding composition or purity was available in the study report (unpublished report, 1989).

The induction was done in two stages: Intradermal injections were performed in the neck region of 20 test animals and succeeded by closed patch occlusive epicutaneous exposure over the injection sites one week later. Induction stage 1: Three pairs of intradermal injections (of 0.1 ml per injection) were made into the neck (shaved) as follows: Adjuvant/saline mixture 1:1 (v/v), test substance in sesame oil (w/v) and the test substance in the adjuvant saline mixture (w/v). The dose level used was 3%.

Induction stage 2: The epidermal induction phase was conducted one week later with the test substance (vaseline was used as the vehicle(w/w)) applied on filter paper to the neck of the animals (patch 2x4 -cm; approx. 0.4 g paste/patch; occluded administration for 48 hours). The concentration used was 10%.

Challenge phase: Two weeks after the epidermal induction application. Animals were tested on the flank with the test substance in vaseline (w/w) and the vehicle alone (patch 2x2 cm; approx. 0.2 g paste per patch; occluded administration for 24 hours). The dose level used was 3%. The challenge reactions were graded after 24 hours and 48 hours according to the Draize scoring scale.

The control group were only treated with adjuvant and the vehicle during the induction periods. During the challenge period the group was treated with the vehicle and with the test substance.

All (20/20) of the tested animals (100%) demonstrated positive dermal reactions when compared with the control group (0/20 positive dermal reactions). The test substance was concluded by the study authors to be an extreme skin sensitiser under the conditions of this study, but due to the relatively high concentration used for the induction phase, in combination with the high incidence of sensitised animals, the CLP criteria are not directly applicable for sub-categorisation of the substance. The eMSCA has evaluated this study as reliable with restrictions, Klimisch 2.

Guinea pig maximisation test (1991)

Another Guinea pig maximisation test was performed in 1991 according to OECD TG 406 (version 1981) with GLP compliance. The test substance was described as o-cresyl-glycidyl-ether (identical to 2,3-epoxypropyl o-tolyl ether) (purity 98.9%, no further information on the chemical identity of impurities was available). The highest non-irritating test article concentration used for the challenge phase was 1%. 10 male and 10 female guinea pigs were used in the test group and 5 male and 5 female guinea pigs in the control group (Unpublished report, 1991a).

Induction stage 1: Three pairs of intradermal injections (of 0.1 ml per injection) were made into the back of the animals: Freund's complete adjuvant 1:1 with bi-distilled water, test

article diluted to 5 % with oleum arachides and the test substance (dose 5%) emulsified in a 1:1 mixture of Freund's complete adjuvant and oleum arachides.

Induction stage 2: The epidermal induction was conducted one week after the intradermal injections: A patch of filter paper was saturated with the test substance (10% in vaseline) and placed over the injection sites of the test animals. The patches were left in place for approximately 48 hours.

Challenge phase: Two weeks after the epidermal induction application, the animals were tested on the flank with the test substance in vaseline (w/w) and the vehicle alone (patch 2x2 cm; approx. 0.2 g paste per patch; occluded administration for 24 hours). The concentration used was 1%. The challenge reactions were graded after 24 hours and 48 hours (14 positive of 20 animals (70%)) according to the Draize scoring scale.

Results: Positive reactions to the challenge 24 hours after treated with the test substance were seen in 16 of 20 animals (80%) and 14 positive reactions were seen 48 hours after challenge (70%). In the negative control group, no positive reactions were observed (0/10). The test substance was considered to be a "strong" dermal sensitizer by the authors of the study under the conditions of the experiment. Because of the relatively high induction concentration of 5%, sub-classification is not possible based on this study. The eMSCA has evaluated this study as reliable with restrictions, Klimisch 2.

Non-guideline study similar to the Guinea pig maximisation test (1976)

A non-guideline study like the Guinea pig maximisation test was performed in 1976 (unpublished report, 1976). The test substance was defined by trade name only (not identified by chemical name or CAS Number and no information was available about purity or chemical identity of impurities). 10 male and 10 female guinea pigs were tested in each group. For the positive control group, a total of 10 animals were tested.

Induction phase: Volumes of 0.1 ml of the test substance (0.1%) in saline without adjuvant were injected intradermally three days during week 1. The test substance was mixed with adjuvant in a 1: 1 ratio. A total of 6 sensitizing doses of 0.1 mL were injected intracutaneously into the skin of the neck during the second and third week of induction.

Challenge phase: Two weeks after the last sensitising treatment with the adjuvant mixture 0.1 mL of the test substance (0.1%) in saline without adjuvant was injected intradermally on the previously untreated flank. The reaction sites were evaluated 24 hours after the challenge by skin-fold thickness determined with a skin—fold gauge: length and height of erythema was recorded and compared to the length, width and height of erythema that occurred after the first week of induction.

In the test group 3 animals out of 20 elicited an erythematous reaction. No erythematous reactions were observed in the negative control group. Dermal reaction scores according to the Magnusson and Kligman scale criteria were not recorded in this study. The eMSCA has evaluated this study as not reliable, Klimisch 3.

Local Lymph Node Assay (LLNA) (2019)

Based on the available data, the eMSCA considered that it was not possible to establish the skin sensitising potency of the Substance EPOTE based on the GPMT data, hence a new *in vivo* study on skin sensitisation, a Local Lymph Node Assay (LLNA) (OECD TG 429), was requested in the ECHA final decision from January 2018. This test was performed in 2019 according to OECD TG 429 (version 2010) with GLP compliance (unpublished report, 2019b). The test substance was described as 2,3-epoxypropylo-tolylether (purity approximately 90%, no further information on the chemical impurities was available). The highest non-irritant test concentration with no signs of systemic toxicity was identified to be 2.5% in a pre-test. Thus, the assay was performed using test concentrations of 0.5, 1, and 2.5% in vehicle acetone: olive oil (4:1, v/v) (AOO 4:1 v/v) with a vehicle control group.

The choice of vehicle is not further justified in the study report, although a justification was requested in the study report. However, since acetone: olive oil is one of the recommended vehicles in the guideline, the eMSCA finds this sufficient.

Preparations of test formulations were made freshly before each application to ensure maximal exposure to unreacted EPOTE. In the ECHA draft decision from January 2018, it was required that homogenecity and stability of the test formulations were analysed and documented in the study report. No such documentation is given in the study report, however, since preparations were freshly made prior to each application, the eMSCA finds this sufficient to ensure adequate EPOTE exposure.

Four female mice of the CBA/CaOlaHsd strain (age 8-13 weeks) were randomly distributed to each group. Each test group was treated by topical application to the dorsal surface of the ear, with 25 µl of the respective test concentrations in AOO (4:1, v/v) on each ear once daily for three consecutive days. The vehicle control group was treated with the equivalent volume of the vehicle alone.

Five days after the first application all animals were injected with ^{3}H-methyl-thymidine (^{3}HTdR) in a phosphate-buffered saline via the tail vein. Approximately five hours after the treatment all animals were euthanized and the lymph nodes were harvested, and the animals were sacrificed.

During the study the animals were observed daily and any signs of systemic toxicity, skin irritation and illness were recorded. Body weights were recorded prior to dosing and sacrifice.

Single cell suspensions of pooled lymph node cells were prepared, and the cellular proliferation were determined by measuring ^{3}HTdR in a β-scintillation counter, expressing ^{3}HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Background levels of ^{3}HTdR were measured.
The proliferative response of the lymph node cells is expressed as DPM per lymph node (mean values) of test animals relative to control animals (Stimulation Index; SI) adjusted for background levels.

If the test concentration results in a 3-fold increase or greater in ^{3}HTdR incorporation (SI of 3) and data has a dose-response relationship, the test is considered positive. The Estimated Concentration of the test substance required to produce a SI of 3 (EC3) was calculated.

Two deviations from the study plan are mentioned in the study report. The age of the mice were 8 to 13 weeks instead of 8 to 12 weeks. The relative humidity in the environment where the mice were kept was for a few hours between approximately 13-45% instead of 45-65%. The authors consider that the deviations did not affect the validity of the study.
A periodic positive control study with α-hexyl cinnamaldehyde was performed using CBA/CaOlaHsd mice in October 2019.
No signs of systemic toxicity or local skin irritation at the ears were observed during the study period. From days 2 and 3 the animals showed an erythema of the ear skin corresponding to score 1 of the test guideline.

The test concentrations of 0.5, 1, and 2.5% resulted in a SI of 1.58, 2.09, and 6.34, respectively. The test concentration of 2.5% resulted in a SI of 6.34 with data having a dose-response relationship, thus EPOTE tested positive for skin sensitising effects. The EC3 value was calculated to be 1.3%, showing that EPOTE is a strong skin sensitiser. The eMSCA has evaluated the study to be reliable without restrictions, Klimisch 1.

7.9.3.2. Human studies

The sensitising properties of the Substance EPOTE have been assessed in the report ‘Ranking of components of epoxy resin systems on the basis of their sensitizing potency’ from the German Forschungs- und Beratungsinstitut Gefahrstoffe (FOBIG, 2012). The

report from 2012 (737 pages) is a thorough evaluation of the use, experimental and human data on the sensitising capacity of epoxy chemicals. Contact allergy against o-cresyl glycidyl ethers have been described in studies of occupational exposure, usually with simultaneous reaction to phenylglycidyl ether:

In one study patch testing was performed in the years 1984 to 1988 on a total of 140 patients suspected of occupational skin disease. Of these, 8 responded positively (5.7%) to a concentration of 0.25% o-cresylglycidyl ether. Details about cross-reactions, of individual exposures or of the clinical relevance of the reactions in the patients with a positive response to o-cresylglycidyl ether are only available for one of the eight patients (Jolanki *et al.,* 1990, reviewed in FOBIG 2012).

In 1997, Kanerva *et al.,* published the results of patch tests (no further details) with 50 substances from a plastic and glue test series. For EPOTE 3 out of 146 patients (2.1%) showed allergic reactions to a concentration of 0.25% o-cresylglycidyl ether. Details from the study were not available (Kanerva *et al.,* 1997, reviewed in FOBIG 2012).

A study by Tarvainen reported results of a plastic and glue test series, conducted in the years 1985 to 1992. Only one of 343 patients had a positive reaction to o-cresylglycidyl ether (0.25%). However, the clinical relevance of this reaction could not be established (Tarvainen 1995, reviewed in FOBIG 2012).

In 1996 Angelini et al. reported a case of contact dermatitis to o-cresyl glycidyl ether in marble workers. 10/22 workers handling a bicomponent resin, based on epoxy resin and o-cresyl glycidyl ether developed contact dermatitis and airborne contact dermatitis within 20 days to 2 months of exposure. When patch tested the 10 symptomatic subjects were all positive to the reactive diluent o-cresyl glycidyl ether and 4 of them also to epoxy resin. Phenyl glycidyl ether also yielded positive responses (in 7/10 cases).

Conclusion of the FOBIG report: In the report EPOTE is categorised as having a high sensitising potency (“HS”).

7.9.3.3. Conclusion on sensitisation

Two reliable Guinea pig maximisation tests have been performed according to OECD TG 406. The results of these studies show that the Substance EPOTE is a strong to extreme skin sensitizer, fulfilling the criteria for category 1 according to CLP. In addition, contact allergy against o-cresyl glycidyl ethers including EPOTE have been described in studies of occupational exposure, pointing to a high skin sensitising potency of the substance.

A LLNA was requested in the ECHA decision from January 2018 to evaluate the skin sensitising potency of EPOTE (unpublished report 2019b). The test was conducted in 2019 and was evaluated by the eMSCA to be reliable without restrictions, Klimisch 1. The study was conducted according to OECD TG 429 and was GLP compliant. The test showed a dose-response relationship with an EC3 of 1.3%. In CLP, skin sensitisers with EC3 values ≤2% are considered strong sensitisers. Thus, according to the CLP criteria, the LLNA points to classification of EPOTE as a strong sensitiser, category 1A.

7.9.4. Repeated dose toxicity

Not evaluated by the eMSCA

7.9.5. Mutagenicity

EPOTE is mutagenic *in vitro* and *in vivo* and has a harmonised classification for mutagenicity as Muta 2, H341; Suspected of causing genetic defects. The substance was included in CORAP with a concern on mutagenicity in germ cells.

7.9.5.1. *In vitro* studies on mutagenicity

Gene mutations in bacteria and yeast

The Substance EPOTE was tested for gene mutations in bacteria and yeast in a study report from 1978 (Unpublished report 1978a).

The test material was tested in the *salmonella* strains TA 1535, TA1537, TA1538, TA98, TA100 and in Saccharomyces cerevisiae D4. Positive concentration-related results were obtained with the test substance in base-pair substitution strains TA1535 both with and without S9 activation and TA100 without rat liver S9 metabolic activation. The eMSCA has evaluated this study as reliable with restrictions, Klimisch 2.

In another study from 1986 EPOTE was tested in the Ames test (OECD TG 471) in the *salmonella* strains TA98, TA100, TA1535, TA1537 and TA97 with and without S9 metabolic activation. The substance caused reproducible gene mutations in TA100 and TA1535 with a dose-related increase without metabolic activation (Canter *et al*., 1986).
The evaluating MSCA has evaluated this study as reliable with restrictions, Klimisch 2.

DNA damage and/or repair study in mammalian cells

An *in vitro* assay investigating unscheduled DNA synthesis (DNA excision repair assessed by amount of incorporated 3H-thymidine) in human lymphocytes was conducted in 1977. The study was not conducted according to any international guidelines. Concentrations of test solution (in DMSO) were 10, 100 and 1000 ug/mL and treatment were for 4.5 hours in triplicate cultures of 1.4 million lymphocytes. At both 10 and 100 ug/mL of the test substance there was a statistically significant ($p < 0.05$) increase of incorporated 3H-thymidine. At 100 ug/mL the increase was approximately 1.5-fold of the mean value of the untreated control. At 1000 ug/mL obvious cytotoxicity was observed as well as a marked reduction in unscheduled DNA synthesis (Unpublished report 1977a).
The evaluating MSCA has evaluated this study as reliable with restrictions, Klimisch 2.

7.9.5.2. *In vivo* studies on mutagenicity in somatic tissue

Transgenic rodent mutagenicity assay (2000)

In 2000 a Transgenic Rodent Mutation Assay was conducted in the MutaMouse™ strain. The study was conducted prior to the adoption of the OECD TG 488 test guideline and according to the following publications: Ashby and Tinwell (1994), and Dean and Mylir (1994). The study was conducted according to GLP (Unpublished report 2000).

Dosing and administration
Dosing preparations were made on each day of treatments in acetone to give the maximum required dosing solution concentration at a dose volume of 2 ml/kg. The test article preparations were protected from light and used within 2¾ hours of initial formulation. Vehicle control was acetone, at a dose volume of 2 ml/kg. The positive control used was Benzo[a]pyrene, which was administered at 0.25 mg/kg bw/day as a solution in acetone (dose volume of 1 ml/kg). Animals were dosed by dermal application to a shaved area of the skin on the back. Evaporation to dryness was permitted. The study report does not state the size of the shaved area of skin.

Range dose finding study
A range-finding study was conducted using groups of three male Muta™ mice dosed with 500, 1000 and 2000 mg/kg bw/day respectively. The two highest dose groups displayed clinical signs of toxicity including swelling of the abdomen, closing of the eyes, opaque eyes, piloerection, l7.9.5.3.ethargy and swollen hind limbs. Animals at all three dose groups displayed signs of significant irritation at the dermal site of administration, including reddening of the dosing site, eschar formation and lightening of the skin. For the two highest doses the irritation of skin was so severe that it compromised the endpoint of skin assessment, this along with the serious systemic effects for the two highest doses resulted in 500 mg/kg bw/day being considered as the maximum tolerated dose and this dose was used in the main experiment. Animals in the lowest dose group (500 mg/kg bw/day) were

dosed once daily for five consecutive days with the test article via dermal application. However, due to the severity of the observed clinical signs, animals in the highest two dose groups (1000 and 2000 mg/kg bw/day) were dosed once daily for only four consecutive days. One animal in the 1000 mg/kg bw/day dose group was killed *in extremis.*

The main study: Experimental setup
In the main study, five male Muta™ mice were included in each group but only one dose group was tested (500 mg/kg bw/day, dose volume 2 ml/kg). The animals were dosed dermally with EPOTE in acetone once per day on each of 5 consecutive days and sacrificed on Day 12 or 33 (7 and 28 days of mutation expression time respectively). Animals in the positive control group exposed to benzo[a]pyrene was sacrificed on day 12. Mutation frequencies (MF) were calculated when plaque forming units (pfu) for each tissue (skin, bone marrow and liver) exceeded 200,000 for most samples. When it was not possible to achieve 200,000 pfu, calculations were conducted for the highest number of pfu available (>120,000 pfu). For one animal in the bone marrow 500 mg/kg bw/day treatment group (sampled on day 33) it was not possible to recover any mutation data, due to extremely low pfu's. Consequently, this test group consisted of only 4 animals instead of 5. Statistical analyses were performed using analysis of variance (ANOVA).

Results of the main study
Treatment at 500 mg/kg bw/day resulted in increases in mutation frequencies that were statistically significant when using ANOVA on both rank-transformed and untransformed data for both bone marrow and liver tissues from EPOTE treated animals sacrificed on day 12. Because of the positive result in bone marrow additional pfu's were collected from the control group and test group sacrificed at day 12. This additional packaging and plating were done to reduce any artefactual variability. The positive result was unchanged after increasing the number of pfu's. The mean bone marrow MF of 64.9 x 10^{-6} (SD 24.3x10^{-6}) at 500 mg/Kg, was statistically significantly different ($p < 0.01$) from the concurrent vehicle control MF mean value of 38.5 x 10^{-6} (SD 4.8x10^{-6}) for bone marrow for un-transformed ($P<0.05$) and rank-transformed data ($P<0.01$). The mean bone marrow MF of 64.9 x 10^{-6} for the treatment group is also higher than the mean MF for historical negative controls (47.9 x 10^{-6}) but may not be statistically significant due to the large standard deviation (48%) for the claimed historical control. There are however only limited data available on the historical controls, and the lack of detail makes the interpretation of the historical controls very difficult and therefore conclusions in this regard uncertain.

The mean mutation frequency for liver tissue for the 500 mg/kg bw/day (sacrificed at day 12) group was 65.9 x 10^{-6} (SD 15.2 x 10^{-6}). Although this is only an increase of 1.26-fold, this result was statistically significantly elevated ($p < 0.05$) from the concurrent vehicle control MF mean value of 52.1 x 10^{-6} (SD 9.2 x10^{-6}) for the liver from animals sacrificed at day 12 when un-transformed data were analyzed (not for transformed data). When data was rank-transformed the increased mutation frequency in the test group was no longer statistically significant. Negative historic control data are available in the study report for bone marrow 47.9 x 10^{-6} (SD 23 x10^{-6}) (N=16) and liver 74.4 x 10^{-6} (SD 24.8 x10^{-6}) (N=24). Mutation frequency in skin samples was not elevated.
This pre-guideline transgenic mouse study was not very sensitive due to the following reasons: The volatility of the test substance combined with the application method and the choice of exposure route (based on toxicokinetic studies dermal absorption is not very high). This makes it uncertain how much of the applied dose was made systemically available. Furthermore, the duration of exposure in this study was insufficient and may have made the study insensitive. Even so, the transgenic mouse study yielded a positive result in bone marrow (distant tissue) for the treatment group at day 12. Moreover, there are indications of an increase in mutation frequency in the liver as well (day 12).
The evaluating MSCA has evaluated this study as reliable with restrictions, Klimisch 2.

Micronucleus assay (1977)

A pre-guideline micronucleus assay with the test material identified as O-cresyl-Glycidyl ether (no information on purity or chemical identity of impurities available). Ten female mice of the B6D2F1 strain were exposed by oral gavage at 125 mg/kg bw/day for 5 days.

The positive control (triethylmelanine) was i.p. injected at 0.5 mg/kg. All animals were sacrificed 4 hours after the last treatment. The details for this study are limited. No information on how many cells were scored per animal is available. Furthermore, no information on changes in PCE/NCE ratio or other indications or other data demonstrating that the bone marrow was exposed under the conditions of this study are available. The test substance did not induce an increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow micronuclei under the conditions of this study (Unpublished report (1977). The evaluating MSCA has evaluated this study as unreliable, Klimisch 3.

Micronucleus assay (1991)

An OECD TG 474 guideline micronucleus assay according to GLP was conducted with the test material identified as O-cresyl-Glycidyl ether (95.3%, no information on chemical identity of impurities is available). Groups of 5 male and 5 female mice of the albino BKW strain were exposed by oral gavage to a single dose of 2000 mg/kg bodyweight. The test material was freshly prepared in a suspension with arachis oil B.P. Groups of ten animals were killed after 24, 48 or 72 hours. The positive control was treated with cyclophosphamide (50 mg/kg bodyweight) and killed 24 hours after treatment. 1000 PCE cells and 1000 NCE cells were scored per animal. The test substance did not induce evidence of chromosome damage in the bone marrow of treated mice under the conditions of the study. The test substance did not induce evidence of cytotoxicity to the bone marrow. There was no significant change in the NCE/PCE ratio in any of the test material treatment groups when compared to their concurrent vehicle control groups or other indications or other data demonstrating that the bone marrow was exposed under the conditions of this study are available (unpublished report 1991b).
The evaluating MSCA has evaluated this study reliable with restrictions, Klimisch 2.

7.9.5.4. Germ cell mutagenicity

Dominant lethal assay (1977)

Dosing administration and experimental setup
This study was performed before the first OECD TG 478 was adopted in 1984 according to the principles in Green et al. 1975. Mice of the B6D2F1 strain were used for the study. Male mice were 8-10 weeks old at the beginning of the study and females were 8-10 weeks old when mated. 10 male mice and 60 female mice were used per group. Male mice had proven fertility (Unpublished report 1977).

24 hours prior to treatment 15-20% of the surface area in the dorsal area of the male mice were clipped by electric shears and remaining hairs were chemically depilated so that no hair remained to interfere with absorption of the test substance. Chemical depilation was only used as needed following the initial removal of hair and did not exceed one depilation per week. According to the study report male mice were exposed to 1.5 g/kg body weight undiluted EPOTE by dermal exposure 3 times a week for a minimum of 8 weeks (only one dose group was tested). The positive control used was Triethylenemelamine (TEM), which was prepared freshly in 0.9% saline and injected once via I.P. at 0.2 mg/kg body weight. Negative controls were sham treated. Following the treatment period 3 untreated nulliparous females were randomly caged per treated male for one week. At the end of the first week the females were replaced with three other untreated virgin females for the duration of the second week.

Female animals were sacrificed 13-14 days from the presumed mating time without being checked for vaginal plugs. At autopsy females were scored for pregnancy, total number of implants and fetal deaths. Statistical comparison between treatment groups and controls were done by analysis of variance. According to the study report the dose was selected based on a range finding study. No further information is available in the study report.

Results
There were no changes in the total number of fetal deaths per pregnancy between the control group and the treated group. When implants per pregnancy were compared between the control group (8.28) and the treated group 2 weeks post treatment (6.97) a

statistically significant reduction was observed (P<0.05). Furthermore, when the treated group was compared to the control group a statistically significant reduction (P< 0.03) was observed in the pregnancy rate of the treated group (week one 75.8%; week 2 63.6%) when compared to the control group (week one 73.4%; week 2 83.5%). Induction of dominant lethal mutations after exposure to test material indicates that the test material has affected the germ cells of the test animal. Dominant lethal mutations are believed to be primarily due to structural or numerical chromosome aberrations even though a mechanism of gene mutation cannot be fully ruled out. However, it is also possible that the induced effect is non-genotoxic. The evaluating MSCA has evaluated this study as reliable with restrictions, Klimisch 2.

Transgenic rodent mutagenicity assay in liver and germ cells (2019)

The study was conducted in male transgenic C57BL/6 Big Blue® mice, and the mutant frequency was assessed at the *CII* locus in liver and testes according to the ECHA substance evaluation adopted decision (2018) (Unpublished report 2019a).

Characterisation and preparation of test material

The identity, strength, purity and composition or other characteristics of the test material was not provided to the Study Director. The purity per protocol was 85.5%. EPOTE was stable in corn oil at concentrations of 0.97 mg/mL for at least 24 hours when stored at room temperature. The stability was 194 mg/mL for at least 9 days when stored at 2-8 °C. Dose formulations were prepared weekly. Corn oil was used as the vehicle based on solubility of EPOTE; a solubility test showed that EPOTE was soluble in corn oil at a concentration of approximately 200 mg/mL. The formulations were stirred until uniform, and the final formulations were determined to be solutions and were stored at 2-8 °C until dosing. All formulations could equilibrate to room temperature, with stirring, for at least 20 minutes prior to dosing. This procedure is assessed to be acceptable to obtain a homogenous solution for dosing. Dose formulations for the main study were analysed for accuracy of concentration by high-pressure liquid chromatography (HPLC).

Dose range finding study

C57BL/6 wild type male mice were used. The animals were exposed to EPOTE via oral gavage for 5 consecutive days. The dose volume was 10 mL/kg. Four groups of five mice in each group were exposed; Vehicle (control group) exposed to only corn oil, 250 mg EPOTE/kg bw/day, 500 mg/kg bw/day and 1000 mg/kg bw/day. The highest dose group animals showed several signs of toxicity during clinical observations such as laboured breathing, ruffled fur, squinty eye and decreased motor activity etc. There were no toxicologically relevant differences in body weights between the dosed grouped animals compared to control animals and all the animals survived.

Experimental setup of the main study

In the main study, male Big Blue® mice were used. Based on the dose-range finding study, the doses for the main study were: 125, 250 and 500 mg/kg bw/day and the vehicle control group only dosed to corn oil. Six animals were dosed in each dose group and tissues from five animals per dose group were prepared and tested for mutant frequency. A concurrent positive control group was not tested. Previously isolated DNA from tissues (liver and testes) collected from six male mice exposed to ethyl nitrosourea (ENU, CAS RN759-73-9) at 40 mg/kg bw/dose, on three consecutive days and necropsied on day 31, was used as a packaging positive control, to confirm the success of the packaging process. These animals were dosed in the study AF57YE.170.BTL. Not using concurrent positive control animals and using DNA from previous positive control treated animals is acceptable according to the OECD TG.

All the animals in the main study were dosed by oral gavage once daily for 28 consecutive days and the dose volume was 10 ml/kg and the sampling time was 28 days (28+28). A full necropsy was performed and the bone marrow, liver testes, duodenum and glandular stomach were collected. Liver and testes from at least five animals/group were processed for DNA isolation and analysed for *CII* mutants. At least 125,000 phages were evaluated from at least two packagings of DNA.

Mutant frequency data analysis of the main study
The dosed groups were compared with the control group using a 1-Way Analysis of Variance (ANOVA) with Dunnett's test as the post hoc test. The positive control group was compared with the control group using a 1-Way Analysis of Variance (ANOVA). Because the ratio is extremely small and may not be normally distributed, a log10 transformation of the MF data was performed.

Results of the main study
All animals survived until termination on day 56. Clinical observations did now show any toxicologically relevant signs. No relevant differences in body weights or body weight gains were observed between the dosed groups and the control group. No relevant observations were observed during gross pathology. In duodenum, glandular stomach and the right testes weight, there were no difference in the organ weights between the dosed groups and the control dose group. In liver weights there was a decrease of 10% the highest dose compared to the control animals. However, the difference was not statistically significant.

Table 9. Results of mutant frequencies in liver and testes

Dose	Liver Mean Mutant Frequency ± SD ($*10^{-6}$)	Testes Mean Mutant Frequency ± SD ($*10^{-6}$)
Control group	26.1±9.7	17.4±5.1
125 mg/kg bw/day	34.0±13	27.0±9.9
250 mg/kg bw/day	29.2±4.3	30.1±25.2
500 mg/kg bw/day	31.4±10.7	22.2±9.2
Positive control ENU[1]	95.3±23.9*	112.8±38.5*

[1]: 40 mg/kg bw/day, dosed on days 1,2 and 3, necropsied on day 31.
*: Statistically significant compared to control group ($p<0.05$).

In the liver, the mutant frequencies of the three doses were not significantly different from the control group and there was no dose response relationship. The positive control gave a significant higher mutant frequency compared to the control group, in the order of 3.7-fold. The mean mutant frequency in the control group (26.1) was lower than the historical mean mutant frequency (46.2). However, mutant frequency data of the individual control animals were within the 95% control limits of the historical control distribution data.

In the testes, the mutant frequencies of the three doses were not significantly different from the control group and there was no dose response relationship. In the lowest dose and mid dose, the mutant frequencies were 1.6 and 1.7-fold higher than the control group, respectively. In both dose groups, it was one animal that caused a higher dose group mean value (low dose: 44.1 and mid dose: 74). By applying Grubbs' method for assessing outliers, both values (44.1 and 74) are calculated to be statistically significant outliers. The positive control gave a significant higher mutant frequency compared to the control group, in the order of 6.5-fold. The mean mutant frequency in the control group (17.4) was comparable to the historical mean mutant frequency (19.1). The study is reliable with restrictions (Klimisch score 2).

7.9.5.5. *In silico* predictions

Predictions for EPOTE were made in the Danish (Q)SAR database (http://qsardb.food.dtu.dk/database/index.html). EPOTE was within the applicability domain and yielded a positive result in a battery of models (CASE Ultra, Leadscope and SciQSAR) for the Ames test in *S. typhimurium*; base-pair Ames Mutagens; chromosome aberrations in Chinese hamster ovary (CHO) cells; mutations in Thymidine Kinase Locus in Mouse Lymphoma cells, mutations in HGPRT Locus in Chinese Hamster ovary cells and Syrian Hamster Embryo (SHE) cell transformation. *In vivo* predictions in the same battery

of models were within the applicability domain and yielded positive results in sister chromatid exchange and in the Comet assay. The micronucleus test was inconclusive and out of domain.

Ashby Structural Alerts for DNA Reactivity

	Battery	CASE Ultra	Leadscope	SciQSAR
Ashby Structural Alerts	POS_IN	POS_IN	NEG_IN	POS_IN

Bacterial Reverse Mutation Test (Ames test)

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Ames test in S. typhimurium (*in vitro*)	POS	POS_IN	POS_IN	POS_IN	POS_IN
- Direct Acting Mutagens (without S9)	NA	INC_OUT	POS_IN	NEG_IN	NEG_IN
- Base-Pair Ames Mutagens	NA	POS_IN	POS_IN	POS_IN	INC_OUT
- Frameshift Ames Mutagens	NA	NEG_IN	NEG_IN	NEG_IN	NEG_IN
- Potent Ames Mutagens, Reversions ≥ 10 Times Controls	NA	NEG_IN	NEG_IN	NEG_IN	POS_OUT

For the four Ames" submodels" (Direct Acting Mutagens (without S9), Base-Pair Ames Mutagens, Frameshift Ames Mutagens, Potent Ames Mutagens) only use the predictions if the main Ames model (Ames test in *S. typhimurium* (*in vitro*)) is POS_IN.

Other *in vitro* Genotoxicity Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells	NA	POS_IN	POS_IN	POS_IN	POS_IN
Chromosome Aberrations in Chinese Hamster Lung (CHL) Cells		POS_OUT	POS_OUT	INC_OUT	POS_IN
Mutations in Thymidine Kinase Locus in Mouse Lymphoma Cells		POS_IN	POS_IN	POS_IN	POS_IN
Mutations in HGPRT Locus in Chinese Hamster Ovary (CHO) Cells		POS_IN	POS_OUT	POS_IN	POS_IN
Unscheduled DNA Synthesis (UDS) in Rat Hepatocytes		NEG_IN	POS_OUT	NEG_IN	NEG_IN
Syrian Hamster Embryo (SHE) Cell Transformation		POS_IN	POS_IN	POS_IN	POS_IN

HGPRT: Hypoxanthine-guanine phosphoribosyltransferase

In vivo Genotoxicity Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Sex-Linked Recessive Lethal (SLRL) Test in Drosophila m.		POS_IN	POS_IN	POS_IN	POS_IN
Micronucleus Test in Mouse Erythrocytes		INC_OUT	INC_OUT	INC_OUT	INC_OUT
Dominant Lethal Mutations in Rodents		INC_OUT	INC_OUT	NEG_IN	POS_IN
Sister Chromatid Exchange in Mouse Bone Marrow Cells		POS_IN	POS_OUT	POS_IN	POS_IN
Comet Assay in Mouse		POS_IN	POS_IN	POS_IN	POS_IN

7.9.5.6. Conclusion on mutagenicity

At present the Substance EPOTE has a harmonized classification for mutagenicity as Muta. 2 according to the CLP Regulation. This classification, which was adopted before the positive result of the dermal TGR study (from 2000) was available, is based on the positive results in *vitro* (Ames TA 100 and TA 1535, UDS in human lymphocytes) and *in vivo* (dominant lethal assay).

In the ECHA substance evaluation adopted decision (2018), a clear concern for gene mutations in germ cells was found. The pre-guideline dermal TGR study from 2000, even though the duration and extent of exposure made the study less sensitive than if it had been performed in accordance with the OECD TG 488, yielded a positive result in bone marrow for the treatment group at day 12 as well as indications of an increase in mutation frequency in the liver at day 12. The recent TGR study (2019) concluded that EPOTE is not mutagenic in germ cells but does not contradict the findings from the previous dermal TGR study from 2000, as only the liver and testes were investigated in the new study and not the bone marrow that was the primary target organ in the dermal TGR from 2000. Therefore, there is no need to revise the harmonized classification as MUTA cat 2.

7.9.6. Carcinogenicity

No carcinogenicity studies have been performed for the Substance EPOTE. Because of strong correlation between *in vivo* mutagenicity and carcinogenicity (Kirkland and Speit 2008), there is a concern that EPOTE may be a genotoxic carcinogen. This is supported by positive QSAR predictions within the applicability domain of all the 7 carcinogenicity models from the Danish (Q)SAR database (http://qsardb.food.dtu.dk/database/index.html). Predictions were made for EPOTE in a commercial MultiCASE CASE Ultra FDA cancer suite consisting of seven models for cancer in male rat, female rat, male mouse, female mouse, rats, mice, and rodents, respectively. All gave positive predictions.

The concern for carcinogenicity could be clarified by performing a carcinogenicity Study in rat, oral route by gavage (OECD TG 451). This study is very time consuming, expensive and uses many animals. As a harmonized classification as Muta 1B entails similar down-stream regulation and risk management measures as a harmonized Carc 1B classification and having regard of, the 3R principles for more ethical use of laboratory animals, the ECHA final decision of 3 January 2018 included a request for an OECD TG 488 in germ cells to first confirm whether EPOTE should be classified as Muta 1B. It was however noted in the decision that if EPOTE would maintain the Muta 2 classification, the need to address the remaining concern for carcinogenicity should be revaluated.

In the follow-up evaluation period, the eMSCA looked to handing over EPOTE to ECHA for a targeted compliance check on the standard information requirements of Annex X, section 8.9.1 of REACH, which gives the Agency the option to require a carcinogenicity study when a substance is classified as germ cell mutagen category 2, and there is widespread dispersive use (see section 7.12). However, although the yearly aggregated tonnage of EPOTE is high (1000 – 10,000 t), the aggregated tonnage is spread out among several registrants and none of the individual registrations are above the 1000 t limit triggering Annex X requirements.

A carcinogenicity study could also be requested in a SEv process based on the concern described above. However, having regard to the costs and animal welfare aspects of a carcinogencity study as well as considerations on proportionality of this possible request, the eMSCA decided at this point to pursue the regulation on the endpoint of skin sensitisation, and conclude the current substance evaluation, thus leaving the carcinogenicity concern unresolved. Subsequently, an internal process at the eMSCA will be initiated to examine whether it will be justified to reintroduce EPOTE to the CoRAP list with a concern for carcinogenicity, based on thorough evaluation of the concern including all relevant information on health hazard, including especially an analysis of the repeated dose toxicity information as well as information on the use pattern of the Substance.

7.9.6.1. Conclusion on carcinogenicity

The concern on possible carcinogenicity of EPOTE due to confirmed *in vivo* mutagenicity in somatic tissue, but not in germ cells, supported by positive QSAR predictions is unresolved. The eMSCA will examine the justification of reintroducing the Substance to the CoRAP list with a concern for carcinogenicity taking the available data, including the repeated dose toxicity and the proportionality principle into account.

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

Not evaluated by the eMSCA

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated by the eMSCA

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

Not evaluated by the eMSCA

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

The requested LLNA Skin sensitisation study, to clarify the potency of the skin sensitisation properties of EPOTE, showed a dose-response relationship with an EC3 of 1,3% Thereby classification of EPOTE as a strong sensitiser, category 1A is justified according to the CLP criteria. As most notifications for classification concerning skin sensitisation, including that of the registrants do not include classification as Skin Sens. 1A, the eMSCA considers that harmonisation of the classification of EPOTE for skin sensitisation is relevant.

With respect to the endpoint of mutagenicity the Substance has a harmonised classification as Muta. Cat 2. The TGR (OECD TG 488) in germ cells requested to clarify whether a Muta. 1 classification could be warranted was negative (Unpublished report, 2019b).
The eMSCAs concern for mutagenicity has therefore been clarified and the available data on mutagenicity supports the current classification as Muta. 2. No further testing on this endpoint is required.
The concern for possible carcinogenicity of EPOTE is unresolved, but no further testing is requested. The available data, including repeated dose toxicity data that was not evaluated in this SEv, potentially justifying reintroducing EPOTE to the CoRAP list with a concern for carcinogenicity, will be thoroughly considered by the eMSCA in a subsequent process.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated by the eMSCA

7.11. PBT and VPVB assessment

Not evaluated by the eMSCA

7.12. Exposure assessment

7.12.1. Human health

The Substance EPOTE is used in products for building and construction work such as adhesives, sealants, coatings, fillers, putties etc. It is also used in the manufacture of plastic products, fabricated metal products, electrical, electronic and optical equipment, machinery and vehicles, rubber products and mineral products. Thus, the Substance is

used by a vast number of sectors with potential of exposure in industrial as well as professional settings. It can thereby be concluded that there is “wide dispersive use” of the Substance according to ECHA guidance chapter R 12.

7.12.1.1. Workers exposure

The endpoint of exposure was selected for substance evaluation of EPOTE among others because RCR values calculated for workers exposure were very high (RCRs in the range of 0.9 – 0.998). Reports on skin sensitisation in workers also verify the potential for exposure in an occupational setting (see section 7.9.3).

The high RCR values reported in previous versions of the Substance CSR were based on DNEL values for inhalation and dermal exposure routes derived from a 21-day inhalation study in rats (Unpublished report 1978b). The observed effects in this study included evidence of nasal tract corrosion and irritation in the high dose group of approximately 305 mg/m^3 together with significant mortality and reduced spermatogenesis in males. This led to a derived NOAEC of 50 mg/m^3 resulting in an inhalation DNEL of 0.46 mg/m^3. The study was given a Klimisch score of 2.

In the newest update of the substance CSR, the RCRs were recalculated using DNEL values derived from an oral exposure 90-days repeated dose toxicity (RDT) study in rats conducted in 2017 according to OECD TG 408 (Unpublished report 2017). In these calculations, an oral NOAEL of 600 mg/kg bw/day was taken as the point of departure resulting in a worker DNEL of 21.12 mg/m^3 for inhalation. When using this DNEL value, the calculated RCR values were no longer in the proximity of 1, and exposure of workers was thereby assumingly much less of a concern. However, the eMSCA is of the opinion that the reliable 21-day inhalation study should be maintained to set the DNEL for inhalation, as the study reflects the relevant exposure route for the worker, and effects relevant for the substance such as corrosion and irritation. Using route to route extrapolation to derive an inhalation DNEL from the oral exposure study is not justified and the reasoning behind doing so has not been adequately explained by the registrant. The DNEL would normally be set on the basis of the lowest NOAEL derived from a reliable and relevant study is ordered to secure the best worker protection.

7.12.1.2. Consumer exposure

According to the registrants, EPOTE is marketed and used in products for industrial and professional use only. Therefore, there are no description of consumer exposure in the substance dossier. However, according to the Nordic product register (SPIN database: http://www.spin2000.net/spinmyphp/) the substance is reported to be used in chemical mixtures targeted for consumers exposure.

EPOTE can also be found in complex articles targeted for consumers, with no release intended such as machinery, mechanical appliances and electrical/electronic products (e.g., computers, cameras, lamps, refrigerators and washing machines). The eMSCA has no data indicating whether residual unreacted EPOTE monomer occurs in end-use products and articles targeted for consumers, but according to the registrant, only very low levels of unreacted EPOTE will be present, and therefore exposure of consumers to EPOTE from these products is not expected.

Based on the information provided in the SPIN database, it is not unlikely that some consumer exposure can occur from e.g., renovation and DIY projects and crafts. While workers can be expected to use personal protection equipment (PPE; in this case gloves, facemask, and protective clothing), it is usually not considered realistic to assume that all consumers will use PPE - even when they are instructed so. Therefore, an occasional exposure of consumers from chemical mixtures containing EPOTE is potentially problematic although it is likely to be less frequent than exposure of workers.

7.12.1.3. Conclusion on human exposure

It can be concluded that there is “wide dispersive use” of EPOTE. As the eMSCA does not agree with using the orally derived DNEL for inhalation exposure scenarios, there is still a concern for high exposure levels of EPOTE. Therefore, the registrant is strongly encouraged to secure that the description of the required PPE provided in the substance CSR, and the information that EPOTE should be marketed for professional use only, is thoroughly passed through the supply chain to achieve acceptable exposure levels of both workers and consumers.

7.12.2. Environment

Not evaluated by the eMSCA

7.13. Risk characterisation

Not evaluated by the eMSCA

7.14. References

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7.15. Abbreviations

ANOVA: Analysis of variance

CLP: Classification, Labelling and Packaging regulation

CoRAP: Community rolling action plan

CSR: Chemical safety report

DD: Draft decision

DMSO: Dimethyl sulfoxide

DNEL: Derived no-effect level

eMSCA: evaluating MSCA

ENU: ethyl nitrosourea

ERC: Environmental release category

GLP: Good laboratory practice

HPLC: high-pressure liquid chromatography

LLNA: Local Lymph Node Assay

EPOTE: 2,3-epoxypropyl o-tolyl ether

MF: Mutant frequency

MSCA: Member state competent authority

NCE: Normochromatic erythrocytes

NOAEC: No observed adverse effect concentration

NOAEL: No observed adverse effect level

PCE: polychromatic erythrocytes

Pfu: Plaque forming units

PPE: Personal protection equipment

PROC: Process category

QSAR: Quantitative structure-activity relationship

REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals

SD: Standard deviation

SU: Sector of end use:

TGR assay: Transgenic Rodent Gene Mutation Assay