Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

2,3-epoxypropyl neodecanoate

EC Number: 247-979-2
CAS Number: 26761-45-5

CLH-O-0000007104-83-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

18 March 2022

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Chemical name: **2,3-epoxypropyl neodecanoate**

EC Number: **247-979-2**

CAS Number: **26761-45-5**

Index Number: **-**

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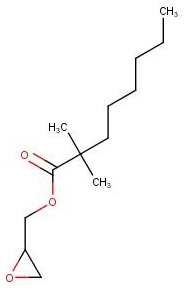
ANNEX 1 - BACKGROUND DOCUMENT TO 2,3-EPOXYPROPYL NEODECANOATE (EPDA)

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1. Substance identity and information related to molecular and structural formula of the substance[1]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	UVCB: (oxiran-2-yl)methyl 2,2-dimethyloctanoate)
Other names (usual name, trade name, abbreviation)	EPDA 2,3-epoxypropyl neodecanoate Glycidyl neodecanoate Neodecanoic acid, oxiranylmethyl ester Oxiran-2-ylmethyl 2-ethyl-2,5-dimethylhexanoate Cardura E10 Cardura E10S Glycidyl Ester of Neodecanoic Acid (GENA) Versatic acid glycidyl ester ECO2200-B ED2800-A-BLACK(E) EH2090PTA-Grey EH2090PTA-Redbrown Shigena-10
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	247-979-2
EC name (if available and appropriate)	2,3-epoxypropyl neodecanoate
CAS number (if available)	26761-45-5
Other identity code (if available)	-
Molecular formula	C13H24O3
Structural formula	

[1] Information on SID source: ECHA Dissemination portal

SMILES notation (if available)	CCCCCCC(C)(C)C(=O)OCC1CO1 O=C((OCC1CO1)C(C)(CC)C(C)CCC
Molecular weight or molecular weight range	228.33 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	No stereo isomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Origin: organic. Manufacturing process is confidential
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant.

1.2 Composition of the substance

Constituents (non-confidential information)

Lists of up to 37 constituents is available in the publicly available registration dossier on ECHA website. Concentration ranges are claimed confidential.
One constituent :1,3-dichloropropan-2-ol (Cas. no. 96-23-1), carries a harmonised classification as Carc 1B, H350, Acute Tox. 3 and Acute Tox. 4 whilst self-classification also includes STOT SE 1/STOT SE 2, Skin Irrit. 2 and Eye Irrit. 2.
The consituent 1-chloro-3-(propan-2-yloxy)propan-2-ol (Cas. no. 4288-84-0) is selfclassified as Acute Tox. 4, Flam. Liq. 4, Skin Irrit. 2, Eye Irrit. 2A and STOT SE 3.
The constituent 2,2'-oxybis(methylene)]bisoxirane (Cas. no. 2238-07-5) is selfclassified as Acute Tox. 4, AcuteTox. 3, Skin Corr. 1B, Acute Tox. 2, Skin Sens. 1, STOT SE 3 and Eye Dam 1.

Test substances

The animal studies referred in this proposal have all been performed with test substances identified by different trade names synonymous with EPDA. The human data on EPDA also represents test performed with trade names synonymous with EPDA. No analytical reports on the tested substances accompany the reports. The registrant has submitted the available animal tests in the registration dossier for EPDA and it is therefore assumed that the tested susbstances are representative of EPDA, although some differences in the exact composition is expected as EPDA is an UVCB.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2. Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	TBD	2,3-epoxypropyl neodecanoate	247-979-2	26761-45-5	Skin Sens. 1A Muta. 2	H317 H341	GHS07 Wng	H317 H341		Skin Sens. 1A; H317: C ≥ 0,001%	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	2,3-epoxypropyl neodecanoate	247-979-2	26761-45-5	Skin Sens. 1A Muta. 2	H317 H341	GHS07 Wng	H317 H341		Skin Sens. 1A; H317: C ≥ 0,001%	-

Table 3. Reason for not proposing harmonised classification and status under public consultation

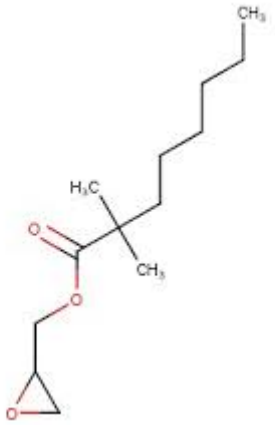
Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation	**harmonised classification proposed**	**Yes**
Germ cell mutagenicity	**harmonised classification proposed**	**Yes**
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure		
Aspiration hazard		
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer		

3 PREVIOUS CLASSIFICATION AND LABELLING

The substance has no harmonised classification in Annex VI of the CLP regulation.

RAC general comment

2,3-epoxypropyl neodecanoate (EPDA) is an unknown or variable composition or biological substance (UVCB) formed by up to 37 constituents according to the publicly available registration dossier on ECHA website. One constituent, 1,3-dichloropropan-2-ol carries a harmonised classification as Carc. 1B, Acute Tox. 3 oral and Acute Tox. 4 dermal; whilst self-classification also includes STOT SE 1/STOT SE 2, Skin Irrit. 2 and Eye Irrit. 2. The constituent 1-chloro-3-(propan-2-yloxy)propan-2-ol is self-classified as Acute Tox. 4, Flam. Liq. 4, Skin Irrit. 2, Eye Irrit. 2A and STOT SE 3. The constituent 2,2'-oxybis(methylene)]bisoxirane is self-classified as Acute Tox. 4 oral, Acute Tox. 3 dermal, Acute Tox. 2 inhalation, Skin Corr. 1B, Skin Sens. 1, STOT SE 3 and Eye Dam 1. The dossier submitter (DS) clarified in the consultation that these three constituents are only present at concentration ranges that would have no influence in the classification. DS also clarified in the consultation that, despite the IUPAC name (oxiran-2-yl)methyl 2,2-dimethyloctanoate) and the structural formula (shown below) referring to only one isomer, the branching of the alkyl chain is highly variable and causes the UVCB nature of EPDA.



Structural formula of EPDA

EPDA is used in adhesives and sealants and has widespread uses across activities and areas by professional workers. DS has used in the CLH report the following data sources: i) publicly available part of the REACH registration dossier and full REACH registration dossier; ii) decision issued by ECHA in the substance evaluation process; iii) public part of the minutes and personal communication with expert at the 51'st Meeting of the Member State Committee; and iv) a search in peer-reviewed scientific literature databases and websites conducted in august 2019 and focused on information published from 2015 to today.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

The substance falls under CLP article 36(1)b (germ cell mutagenicity), for which classification should be harmonised. No justification is needed.

With respect to the end point of skin sensitisation, the substance falls under article 36 (3). The DS wishes for a harmonisation of the classification, as he evaluates that the self-classifications of the substance underestimates the severity of the hazard.

Further detail on need of action at Community level:

The DS' evaluation shows that the available data on skin sensitisation fulfill the criteria for classification as an extreme skin sensitiser and that EPDA thus should be classified as Skin Sens. category 1A with a specific concentration limit (SCL) of 0.001%.
All registrants and most notifiers (1138) selfclassify EPDA as a skin sensitiser. One group of 44 notifiers (total number of notifiers is nearing 1200) has proposed to classify EPDA as Skin sens category 1A, with the general concentration limit (GCL) of 0.1%.
Harmonisation of the classification for skin sensitisation is therefore necessary to secure that European users of EPDA receive sufficient information through the label and through the Safety Data Sheet (SDS) to take relevant precautions in the handling of mixtures containing EPDA at a concentration that may entail sensitisation.

Denmark is the evaluating Member State under substance evaluation of EPDA, and has recently concluded the Follow-Up phase following a request in the ECHA substance evaluation decision from 2016 for a Transgenic Rodent Assay. The evaluating Member State is currently preparing a conclusion document on the substance, as no further information is needed to permit hazard and risk assessment on the end-points of concern raised in CoRAP, i.e. mutagenicity and skin sensitisation.

During the substance evaluation, the data available in the REACH registration dossier on skin sensitisation showed that EPDA is a skin sensitiser with a high potency. The decision from the MSC (October 2016) specified recommendations regarding the data on skin sensitisation:
"It is however important to specify that the concern for skin sensitisation is maintained due to inconsistency between the available data and current self-classification. Further action may be considered to ensure an adequate risk management of the substance (including its classification)."

The DS has scrutinised all available data relevant to the end-point of skin sensitisation, including data from a literature search. On that basis, the DS has prepared the present proposal for a harmonised classification for EPDA as Skin Sens. cat 1A with a SCL of 0.001%.

5 IDENTIFIED USES

The substance is used in adhesives and sealants and has widespread uses across activities and areas by professional workers. ECHA has no publicly registered data indicating whether or in which chemical products the substance might be used for consumer uses (ECHA webpage, Sept 19).

6 DATA SOURCES

The primary source of information is the publicly available part of the REACH registration dossier for EPDA (ECHA webpage, Sept. 2019) and the REACH registration dossier (May 2019). Furthermore the decision issued by ECHA in the substance evaluation process (ECHA, 2017), the public part of the minutes and personal communication with expert at the 51'st Meeting of the Member State Committee (Dec. 2016), is also used as sources.

In addition a search in peer-reviewed scientific literature databases and websites (grey literature) was conducted. The literature search was conducted in august 2019 and focussed on the the period from 2015 to ensure that potentially relevant information published after the substance evaluation was conducted are taken into account. The literature search was conducted using several synonyms and numerical identifiers for EPDA.

The searches have included literature databases such as Google Scholar, PubMed, Web of Science as well as searches in sources such as OECD SIDS and IPCS INCHEM. General searches via Google have also been carried out. For identification of information from grey literature, the OpenGrey database was checked. The OpenGrey is a system for information on grey literature in Europe. However, there were no hits on any searches on EPDA and its related terms.

The search identified five articles with human patch testing in differing contexts relevant for this evaluation of skin sensitising potency for EPDA.

7 PHYSICOCHEMICAL PROPERTIES

Table 4. Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	REACH registration dossier	-
Melting/freezing point	-68ºC	REACH registration dossier	-
Boiling point	269-272 ºC	REACH registration dossier	-
Relative density	958	REACH registration dossier	-
Vapour pressure	1.5 Pa (298 K)	REACH registration dossier	-
Surface tension	50 nM (20ºC and 63mg/L)	REACH registration dossier	-
Water solubility	70 mg/L (20ºC)	REACH registration dossier	-
Partition coefficient n-octanol/water	Log K_{OW} 4.4 (20 ºC)	REACH registration dossier	-
Flash point	126ºC	REACH registration dossier	-
Flammability	-	REACH registration dossier	Not technically feasible

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties	-	REACH registration dossier	No explosive functional groups and oxygen balance less than -200
Self-ignition temperature	276 ± 5 ºC	REACH registration dossier	-
Oxidising properties	-	REACH registration dossier	The study is waived
Granulometry	-	REACH registration dossier	The study is waived
Stability in organic solvents and identity of relevant degradation products	-	REACH registration dossier	The study is waived
Dissociation constant	-	REACH registration dossier	DEHA does not contain functional groups subject to dissociation, consequently a study is not justified.
Viscosity	8.3 mm²/s (20ºC)	REACH registration dossier	-

8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The registration dossier included information from in vitro metabolism studies conducted with cell-free tissue preparations from human, rat and mouse, liver, lung and skin. Detoxication is stated to be rapid, and the predominate pathway of detoxication is considered to be epoxide hydrolase and carboxylesterase hydrolysis to glutathione conjugation. Based on scaling in vitro kinetic data, the registrant states that clearance in humans is approximately an-order-of-magnitude slower relative to rodents.

The dermal penetration and metabolism of radio-labeled 2,3 -epoxypropyl neodecanoate isomer was assessed in vitro in skin samples in rats, mice and humans. The substance was shown to metabolize in vitro to the corresponding diol and ester hydrolysis product. Human skin samples were approximately an order of magnitude less permeable to the 2,3 –epoxypropyl neodecanoate isomer than rodent skin. The mean percent penetration of the 2,3 -epoxypropyl isomer in human skin samples was 0.24% +/- 0.06%.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Hazard class not assessed in this dossier

10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier

10.3 Acute toxicity - inhalation route

Hazard class not assessed in this dossier

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier

10.7 Skin sensitisation

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

10.7.1.1 Animal data

Table 5. Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, (purity)**	**Dose levels** **Induction/ challenge**	**Results Sensitised/ tested** (at 48 h, if not specified); **% of positive testanimals**	**Reference**
GPMT M&K (conducted prior to OECD TG)	Guinea pig P strain 10 female and 10 male test 10 controls	Cardura E10[1] (purity not specified)	0.5 %/ 50 %	19/20; 95 %	Unpublished report, 1977a
GPMT M&K (conducted prior to OECD TG)	Guinea pig P strain 10 female and 10 male test animals 10 controls	Cardura E10 Stripped[2] (purity not specified)	0.05 %/ 50 %	13/20; 65 %	Unpublished report, 1977b
GPMT M&K OECD 406	Guinea pig Dunkin-Hartley 20 test females 10 controls	Cardura E10S[3] (In solvent Alembicol D) (purity not specified)	25 %/ 50 and 25 %	50% challenge: 9/20; 45 % 25% challenge 4(+2 inconclusive); 25-30%	Unpublished report, 1998
GPMT M&K (Insufficient study detail to determine Guideline and GLP)	Guinea pig, Information on strain, sex, numbers/group not available	EPDA (purity not specified)	5 %/50 %	85 %	Unpublished summary, 2003

[1] Cardura E10 is a trade name for EPDA

[2] Cardura E10 Stripped sample has been stripped with nitrogen at 120 ºC to remove contaminants resulting in a total weight loss of 1 %).

[3] The registrant included the study in the registrant as representative for EPDA. However, no information was available on the possible variation in composition from "EPDA".

Four *in-vivo* tests performed with EPDA have been identified.

Two tests were performed in 1977 prior to the establishment of the first OECD guidelines.

A study in Guinea Pigs from 1977 (Unpublished report, 1977a) reports sensitisation response at challenge in 19/20 animals (95 %), following an intradermal induction concentration of 0.5 % EPDA in corn oil, using adjuvant. followed by topical application. The challenge concentration was 50 %. The study design is comparable with the OECD TG 406. The study is assessed to be reliable with restrictions (Klim. 2).

In another Guinea Pig study (Unpublished report, 1977b), 13/20 animals (65%) reacted at first reading 24 hours post challenge. The intradermal induction concentration was 0.05 %. using adjuvant and subsequent topical application, and the challenge dose was 50 %. The test substance has undergone a "stripping" process with nitrogen at 120 °C to remove contaminants, resulting in a total weight loss of 1 % of the tested substance. The vehicle used was corn oil. The description and reporting is clear. The study is assessed as reliable with restrictions (Klim. 2).

Another test (Unpublished report, 1998) was performed in 1998 according to OECD TG 406 (OECD TG as revised in 1992), including intradermal induction with 25% EPDA, topical induction and challenge with 25 and 50% EPDA, at the anterior and posterior part of the back of the guinea pigs. Although there are some unclarities in the scoring of the response in the study report, the author reported that 9 out of 20 Dunkin-Hartley guinea pigs (45 %) showed a positive reaction at 48 hours post-challenge at the challenge concentration 50 %, after an injection of an intradermal induction concentration of 25 %. The reponse at 48 hrs, after 25% challenge is 6 sensitised animals out of 20, 2 of which are reported to be doubtful. The study is assessed as reliable with restrictions (Klim. 2).

An unpublished Guinea Pig Maximisation test was performed in 2003 (Unpublished Study report, 2003). The study was only available to the DS as a summary, hence the Klimish 4 scoring. The summary states the study to be an OECD 406/GLP study. Skin reaction at 48 hrs in 17 out of 20 animals (85%) with an induction concentration of 5 % while the challenge concentration of 50 % was reported. The study concluded that "2,3 - epoxypropyl neodecanoate is a Strong to Extreme skin sensitizer under the conditions of the study".

Overall, the available animal studies on EPDA show that EPDA has elicited a moderate to extreme positive reaction in 4 skin sensitisation tests in Guinea pigs.

10.7.1.2 Human data

Table 6. Summary table of human data on skin sensitisation

Type of data/report	Test substance, (purity)	Relevant information	Observations/Results	Reference
Clinical case study	Cardura E10[1] (purity not specified)	A severe case of dermatitis in a man aged 16 working for 9 days with undiluted epoxy resins. He showed a positive patch test to Cardura E down to 0.01 % in acetone. He also reacted to epoxy resin of the bisphenol A type (0.001 %), but tested negative to isophoronediamine, triethylhexamethylenediamine, N-ethyl o- and p-toluene sulphonamide, and to three different modified polyamidoamine hardeners.	One positive patch-test	Dahlquist et. al., 1979
Clinical case study	Cardura E10[1] (purity not	A 33-year old man working for 3 to 4 years in a polymer plant developed a rash after 6 to 7 days of working with	One positive patch-test 4 negative	Lovell et. al., 1984

Type of data/report	Test substance, (purity)	Relevant information	Observations/Results	Reference
	specified)	epoxy resin in an open tank with Cardura E10 and other reactive diluents and fillers . The patient demonstrated an isolated Cardura E10 sensitivity when patch tested with a concentration of 1% Cardura E10. 4 other workers at the plant were patch tested. Of these 2 were tested positive to epoxy resin, but none were tested positive to Cardura E10. Cardura E10 tested negative in 10 unexposed subjects.	10 controls	
Clinical case study	Cardura E10[1] (purity not specified)	3 female workers in a brush factory developed contact allergy to resin component and 1,4-butanediol diglycidyl ether (BDDGE). A standard series and a plastic and glue series were tested along with a number of dilution series and also specific reactive diluents including Cardura E10 from Shell Chemie, the Netherlands (0.25 % in petroleum).	All three patients presented a negative patch-test for Cardura E10.	Jolanki et. al., 1987
Retrospective study of selected patients from occupational health clinic	Cardura E10[1] (purity not specified)	The patch test was performed with a special epoxy compound series. The patch tests were performed according to International Contact Dermatitis Research Group (ICDRG) recommendations. The article includes information on test substances and their providers. This included Glycidyl ester of neodecanoic acid (Cardura E 10). As a measure of general exposure, the study used information from the Finnish Product Register.	39/39 patients negative to patch-test with 0.25 % dose. 215/215 patients negative to patch-test with 1 % dose. The Product Register had information that there were 99 products on the Finnish market containing Cardura E 10.	Alto-Korte et. al., 2015
Clinical study of diagnostics with selected patients	Versatic acid glycidyl ester[2] (purity not specified)	To improve diagnostics in patients with presumed allergic contact dermatitis due to Epoxy Resin System (ERS), a multicentre study EPOX 2002 was performed. The study included the substance Versatic acid glycidyl ester[1] used in patch test in the concentration 0.25 % in petroleum.	85/87 patients tested negative to patch-test with 0.25 % dose and 2/87 could not be scored. The authors concluded for a number of substances where no reaction was observed, that the test concentration may have been too low to trigger a reaction and they recommend in future studies that it be increased.	Geier et.al., 2004

[1] Cardura E10 is a trade name for EPDA

[2] Versatic acid glycidyl ester*:* carries the same CAS no. as EPDA

EPDA has been included as a constituent in the test material used for for patch testing epoxy resins at the workplace for a number of years.

In a case study reported as a short communication by Dahlquist and co-workers a young man of 16 years of age reacted to 0.01% EPDA after having been working for 9 days filling drums with epoxy resins and reactive diluents (Dahlquist et. al., 1979).

In another short communication, Lovell and co-workers described a case of sensitisation to EPDA following occupational exposure to epoxy resins from working with mixing of epoxy resins with EPDA and other resin chemicals in an open tank for 6-7 days. An itching and papular rash of the forearm, with erythema of the face and swelling of the eylids was recurrent after consecutive exposures. The patient reacted clearly in a patch test to 1% EPDA in petrolatum and midly to 2% resin 4 days after application. Two out of four other workers from the plant (no description of their exposure situation given) reacted to epoxy resins, but not to EPDA specifically. The study further included 10 control subjects, also negative to patch testing with EPDA (Lovell et. al., 1984).

The third study reported negative patch tests to the substance in three workers in a brushfactory who were sensitised to a two-component epoxy-based glue. The workers reacted to the resin component and to other reactive diluents (e.g.1,4-butanediol diglycidyl ether (BDDGE)) (Jolanki et. al., 1987).

In a recent retrospective study a total of 39 selected patients with contact dermatitis occupationally exposed to resins were tested with patch test with EPDA at a concentration of 0.25 %. A further 215 selected patients were patch tested with a concentration of 1 %. No patients in the study reacted to the substance (Alto-Korte et. al., 2015). However no details were available on the occupational exposure levels of EPDA.

In another study also on selected patients with contact dermatitis (Geier et.al., 2004), 87 persons were patch tested with EPDA. Two had a ambiguous reaction (not positive or negative) and 85 tested negative. The test group consisted of patients who had an occupational or non-occupational exposure to epoxy resin systems, which may have included EPDA, but details on exposure to EPDA specifically were not reported. The authors state that the concentration used for patch testing, 0.25%, may have been too low to trigger a response.

The human data on the sentising potential of EPDA is limited. Although the substance has been included in testing for sensitisation to resins at the workplace, reported data on testing of EPDA alone are scarce, and the information on the exposure levels to EPDA at the workplaces is lacking. The human data are overall negative, with only two cases with of sensitisation published. However, the concentration used for patch testing of were relatively low. Overall, the information from these data in humans do not allow for a further assessment of the sensitising potency or subsequent subcategorisation of EPDA.

10.7.1.3 Other data

In a review report by Fobig et. al. (2012) on the sensitisation potential of a number of epoxy hardeners, EPDA was assigned to the category of low to moderate sensitising potency. Fobig and co-workers considered that no conclusion can be drawn on the available human data with respect to sensitisation potency of EPDA due to lack of quantitative data were available to them. The dossier submitter notes that there are some discrepancies in the reporting of some of the animal data included by Fobig and co-workers: Only one study from 1977 is referred to, with the information that the induction dose is 50%. This would appear to be the challenge concentration (which was also referred by the registrant) rather than the intradermal induction dose included in the original two study reports on GPMT conducted in 1977 available to the dossier submitter, which use 0.5 and 0.05% as intradermal induction concentrations, respectively. Frobig et al. further refer a Guinea pig tests conducted in 1998 using an induction concentration of 50%. However, that GPMT used 25% for intradermal induction. Finally, Frobig and co-workers did not include the 2003 GPMT. Therefore, the dossier submitter has not considered the Fobig et al. paper in the weight of evidence evaluation of the data on the sensitising potential of EPDA.

10.7.2 Comparison with the CLP criteria

Classification as a skin sensitiser is warranted when there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons or if there are positive results from an appropriate animal test. The information should be considered in a weight of evidence approach.

The human evidence is scarce and details on the conditions of exposure to EPDA were not available. However, two positive cases in patch testing indicate that EPDA may sensitise humans. Animal data show that EPDA has elicited a moderate to extreme reaction with at least 30% of the animals reacting at challenge in four skin sensitisation maximisation tests in Guinea pigs (GPMT). Therefore, EPDA should be classified for skin sensitisation.

Classification for skin sensitisation should further include subcategorisation in subcategories 1A or 1B when data fulfil cut-offs indicated in the CLP criteria.

As the limited dataset from human patch tests with EPDA does not include information of exposure levels to the substance at the workplace, these data cannot be used for subcategorisation. Thus, subcategorisation of EPDA is based on the available animal studies.

The criteria for for subcategorisation in 1A on the basis of results from GPMT are:
≥ 30 % responding at ≤ 0.1 % intradermal induction dose or
≥ 60 % responding at an intradermal induction dose between 0.1 < and ≤ 1 % .

One study (Unpublished Study report, 1977a) showed that a 0.5 % intradermal induction concentration resulted in 95 % (>60%) sensitised animals. Another study (Unpublished Study report, 1977b) a 0.05 % intradermal induction concentration led to a 65 % (>30%) response in the test animals. DS therefore concludes that both animals studies support classification of EPDA as skin sensitising in sub-category 1A.

In the two further Guinea Pig Maximisation Tests from 1998 and 2003 (Unpublished Study report, 1998; Unpublished Study report, 2003), intradermal induction levels of 25 % and 5 % gave rise to 45% and 85% sensitised animals, respectively. Both tests thus fullfill the criteria for category 1B in having more than *≥ 30 % sensitised following an intradermal induction dose > 1 %* . The relation between induction level and response in the 1998-study does not indicate a high potency of EPDA. However, as the the response in the study from 2003 is very high, subcategorization in category 1A is cannot be excluded on the basis of that study.

Overall, the animal data therefore support classification of EPDA in subcategory 1A according to the CLP criteria.

The guidance on CLP criteria for category 1A sensitisers includes a distinction based on potency between strong and extreme sensitisers leading to the setting of specific concentration limits:
≥ 60 % responding at ≤ 0.1 % intradermal induction = extreme potency (1A) – SCL 0.001% w/v
≥30 - <60 % responding at ≤ 0.1 % intradermal induction = Strong potency (1A) GCL 0.1% w/v
≥ 60 % responding at > 0.1% and ≤ 1.0 % intradermal induction = Strong potency (1A) GCL 0.1% w/v

Results from the one of the GPMT studies (Unpublished Study report, 1977b) which used an intradermal induction concentration of 0.05% (<<0.1%) and resulted in 65% sensitised animals, fulfils the criteria for the potency category "Extreme skin sensitiser". The regime and results in another GPMT study (Unpublished Study report, 1977a) does not exclude extreme sensitising potency of EPDA since almost 100 % of the tested animals were sensitised (95%) at an intradermal induction concentration of 0.5%. The GPMTs from 1998 and 2003, respectively, both use too high induction concentrations to permit evaluation of extreme potency, although they do suggest a lower potency of EPDA.

In summary, the results from four positive Guinea pig maximisation tests support classification of EPDA as a skin sensitiser whilst the information from humans is limited. Two of the animal tests support classification in category 1A and the remaining two studies do not contradict this conclusion.

As EPDA caused very high sensitisation responses in two out of four guinea pig studies which used low induction concentrations, the DS evaluates EPDA to be a skin sensitiser of *extreme potency*, and a specific concentration limit (SCL) of 0.001 % is warranted according the CLP criteria.

10.7.3 Conclusion on classification and labelling for skin sensitisation

EPDA should be classified as Skin sens. Cat 1A with the specific concentration limit (SCL) of 0.001 %. The corresponding hazard statement is H317: May cause an allergic skin reaction.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed classification of EPDA as Skin Sens. 1A and hazard statement H317 (May cause an allergic reaction). The proposal is based on four guinea pig maximisation test (GPMT), two of them warranting category 1A for EPDA and the other two do not contradicting this classification. Moreover, two of these GPMT showed an extreme potency for EPDA; which allowed the DS propose a specific concentration limit (SCL) of 0.001%.

Comments received during consultation

Two different member state competent authorities (MSCA) and one company manufacturer based in the United States of America agreed with the DS's proposal for classification as Skin Sens. 1A. One MSCA requested clarification about the Unpublished report dated on 1998 since according to the CLH report, the results of this study do not contradict subcategorization within 1A although it is actually considered that these results do not support subcategory 1A. The DS replied that indeed the cited study fulfils the criteria for subcategorization in 1B due to the dose used but considering the potential variability of composition in a UVCB, studies with more severe results should be given more weight in the evaluation of relevant SCLs for the substance.

One MSCA argued that experimental results in animals, together with the rather negative results in humans suggest that a general concentration limit of 0.1% would be more appropriate than the proposed SCL of 0.001%. One company manufacturer supported this position and proposed to leave the harmonisation of the classification of EPDA in stand-by and initiate a series of *in silico*, *in vitro* and/or *in vivo* studies including but not limited to the following: OECD TG 442C *In Chemico* Skin Sensitisation; OECD TG 442D ARE-Nrf2 Luciferase Test Method; OECD TG 442A Local Lymph Node Assay: DA; OECD TG 442B Local Lymph Node Assay: BrdU-ELISA or –FCM and OECD TG 429 mouse Local Lymph Node Assay. The DS replied that available *in vivo* animal data were deemed by the REACH registrant sufficient to fulfil REACH requirements and the DS therefore uses the data for classification purposes. The DS underlined that the classification should not be postponed. However, the DS is open to reconsider classification and/or to derive SCLs would substantial new data be provided.

Assessment and comparison with the classification criteria

The animal database contains four GPMT summarised in Table below. All these four studies demonstrated skin sensitising potential for EPDA. Two studies (both Klimisch score 2) showed extreme sensitising potential causing positive results in 95% and 65% of dosed animals after induction with 0.5 and 0.05%; respectively. A third study with Klimisch score 2 showed that EPDA induced skin sensitisation in 45% of animals induced with intradermal injection of 25% of test substance. Finally, the less reliable study (Klimisch score 4) showed

that EPDA induced skin sensitisation in 85% of animals induced with intradermal injection of 5% of test substance. Overall, the available animal studies show that EPDA has elicited a moderate to extreme skin sensitisation in 4 GPMT.

Table: *Summary of the animal study on skin sensitisation with EPDA*

Study	Dose level	Results	Reference
GPMT M&K Comparable with OECD TG 406 Guinea pig P strain: 10 female + 10 male test 10 controls Cardura E101 (trade name for EPDA) Purity not specified	Induction: 0.5% Day 1: Intradermal injection: Two rows of three injections Day 7: Occluded patch for 48 h Challenge: 50% Day 21-24: Topical application. Controls received Freund's complete adjuvant No positive controls	19 (10 males and 9 females) out of 20 animals (95%) showed erythema or severe erythema persisting 48 h after removal of topical challenge patch One control animal showed signs of erythema No signs of systemic toxicity	Unpublished report, 1977a Klimisch score: 2
GPMT M&K Conducted prior to OECD TG Guinea pig P strain: 10 female + 10 male test 10 controls Cardura E10 (trade name for EPDA) (stripped with nitrogen at 120 °C to remove contaminants resulting in a 1% weight loss) Purity not specified	Induction: 0.05% Day 1: Intradermal injection: Two rows of three injections Day 7: Occluded patch for 48 h Challenge: 50% Day 21- 24: topical application. Controls received Freund's complete adjuvant No positive control group was used	13 (5 males + 8 females) out of 20 animals (65%) showed erythema or severe erythema persisting 24 h after removal of challenge patch 7 (2 males + 5 female) out of 20 animals (35%) still showed erythema persisting after 48 h The test animals showed no signs of systemic toxicity No controls showed signs of erythema	Unpublished report, 1977b Klimisch score: 2
GPMT M&K OECD TG 406 Guinea pig Dunkin-Hartley: 20 test females + 10 controls Cardura E10S3 (trade name for EPDA) in solvent Alembicol D Purity not specified	Induction: 25% Day 1: Intraperitoneal injection Day 7: Topical application Challenge: 25 and 50% Day 21: topical application	The test animals showed no signs of systemic toxicity Control animals: Desquamation Slight erythema in 4 animals (after 50% challenge) at 24 and at 48 h after challenge Slight erythema in 2 animals (after 25% challenge) which persisted in one of the animals Exposed animals:	Unpublished report, 1998 Klimisch score: 2

		9/20 (45%) test animals at 50% challenge had individual responses after 48 h 4/20 animals (20%) +2 ambiguous results (30%) gave a positive response to the 25% challenge	
GPMT OECD TG 406 Guideline GLP Guinea pig female: 20 test and 20 control animals EPDA in Drakeol 19 (no CAS and no purity reported)	Induction: 5% Day 1: Intradermal injection Day 7: Topical application Challenge: 50% Day 21 No positive controls	17 animals out of 20 (85%) showed a positive reaction 48 h after challenge	Unpublished summary, 2003 Klimisch score: 4 Only the study summary has been made available to the DS

The CLH report contains data on five studies with humans after occupational exposure (a sixth study was not considered by DS due to inconsistencies). These studies were summarised in Table below; where it is seen that the human data on sensitising potential of EPDA is limited and the information on the exposure levels to EPDA at workplaces is lacking. Overall, the human data were negative, with two positive cases with patch testing with relatively low EPDA concentration.

Table: *Summary table of human data on skin sensitisation.*
Cardura E10 is a trade name for EPDA. Versatic acid glycidyl ester carries the same CAS number as EPDA.

Type of data/report	Test substance	Results	Reference
Clinical case study	Cardura E10 (purity not specified)	One positive patch-test (0.01% in acetone) in a case study report	Dahlquist *et al.*, 1979
Clinical case study	Cardura E10 (purity not specified)	One positive patch test (1%) 4 negative 10 controls	Lovell *et al*., 1984
Clinical case study	Cardura E10 (purity not specified)	3 patients presented a negative patch-test	Jolanki *et al*., 1987
Retrospective study of selected patients from occupational health clinic	Cardura E10 (purity not specified)	39/39 patients negative to patch test with 0.25% dose. 215/215 patients negative to patch-test with 1% dose	Alto-Korte *et al*., 2015
Clinical study of diagnostics with selected patients	Versatic acid glycidyl ester (purity not specified)	85/87 patients tested negative to patch-test with 0.25% dose and 2/87 could not be scored	Geier *et al*., 2004

Comparison with the criteria

The human data are scarce and there are gaps as regard as exposure conditions. However, the database contains two positive cases in patch testing indicating that EPDA may sensitise humans (Table above). Animal data show that EPDA is able to elicit skin sensitisation in more than 30% of animals in four GPMT. Overall, based on animal data, RAC notes that EPDA should be classified as skin sensitiser.

Human data do not allow subcategorization since information about real occupational exposure is lacking. Thus, the subcategorization should rely on animal data. The criteria for subcategorization based on results from GPMT are as follows:

- Subcategory 1A: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at an intradermal induction dose between 0.1 < and ≤ 1%
- Subcategory 1B: ≥ 30% to < 60% responding at > 0,1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose

Two of the available studies (Unpublished reports 1977a and 1977b) would warrant classification within subcategory 1A since 95% and 65% of sensitisation were noted after intradermal inductions of 0.5% and 0.05% EPDA; respectively (see Table above on animal data). A third study (Unpublished summary, 2003) would warrant classification within subcategory 1B since 85% of sensitisation was reached after an intradermal induction of 5% EPDA. However, RAC notes that concentrations lower than 1% were not tested during the induction and therefore this study does not allow ruled out subcategory 1A. Finally, the Unpublished report (1998) reported 45% sensitisation after induction with 25% EPDA; which would also warrant classification within subcategory 1B. However, it is noted by RAC that in this fourth study the induction was performed through intraperitoneal injection instead of intradermal injection. Thus, this study is used in the weight of evidence for supporting the classification but is not used by RAC for setting the subcategorization. Overall, based on weight of evidence in animal data, RAC notes that the classification of EPDA in subcategory 1A is warranted.

The CLP criteria for distinction of sensitisation potency is summarised below:

Concentration for topical induction (% w/v)	Incidence sensitised guinea pigs (%)	Potency	Resulting subcategory
≤0.1	≥60	Extreme	1A
≤0.1	>30 - <60	Strong	1A
>0.1 - ≤1.0	≥60	Strong	1A

The results of the Unpublished report (1977b) fit within extreme potency since 65% of sensitisation was reached with a topical induction of 0.05%. On the other hand, the Unpublished report (1977a), with 95% of sensitisation after topical induction with 0.5% EPDA would support a strong potency; while the other two studies use too high induction concentration to permit assessing potency. RAC notes that the Unpublished report (1997a) caused almost 100% sensitisation with 0.5% topical induction and the percentage of animals that would have been sensitised with an intradermal induction lower than 0.1% still could be higher than 60%. Therefore, this study points towards strong potency but does not allow rule out extreme potency. Overall, in a weight of evidence approach, RAC proposes the classification of EPDA as extreme skin sensitiser.

In conclusion, **RAC supports the DS's proposal for classification of EPDA as Skin Sens. 1A with SCL of 0.001% and hazard statement H317 (may cause an allergic skin reaction).**

10.8 Mutagenicity

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

10.8.1.1 Animal data

10.8.1.1.1 *In vitro* data

Table 7. Summary table of mutagenicity/genotoxicity tests *in vitro*

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) OECD Guideline 471 (Bacterial Reverse Mutation Assay) Klim:1 (reliable without restriction)	Test material (EC name): 2,3-epoxypropyl neodecanoate Form: liquid at room temp. (Purity not specified).	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100	Test concentrations: 1.6, 8, 40, 200, 1000, and 5000 ug/plate for the 1st mutation study. 125, 250, 500, 1000, 2000, and 5000 ug/plate for the 2nd mutation study. Both trials conducted with and without rat liver dervide S9 metabolic activation preparation. Positive control substance(s): sodium azide (2-NF, 9-aminoacrimide GLU , 2- anthramine) (met. act.: with and without)	Evaluation of results: **Positive with metabolic activation** Test results: positive for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested) with S9 metabolic activation. cytotoxicity: yes (between 1000 and 5000 ug/plate.) ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	Dawkes (1998)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Test material (EC name): 2,3-epoxypropyl neodecanoate Purity:epichlorohydrin content 0.096% and >5ppm. Form: Liquid at	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 and Escherichia coli strains	Test Concentrations: 0, 0.2, 2, 500 and 2000 ug per plate. (met. act.: with and without)	Evaluation of results: **Positive with metabolic activation** Test results: positive (With rat liver S-9 metabolic	B. J. Dean, T.M. Brooks, G. Hodson—Walker, and G. Pook

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
Klim: 2 (reliable with restriction).	room temperature.	WP2 and WP2 uvrA	Positive control substance(s): benzo(a)pyrene (Benzo(a) pyrene was used with S-9 metabolic activation and 4-nitroquinoline oxide was without S-9 mix.)	activation preparation.) for Salmonella typhimurium TA 1535, TA 1538, TA 98 and TA 100 and Escherichia coli strains WP2 and WP2 uvrA.(all strains/cell types tested) with S9 metabolic activation. negative controls valid: not applicable; positive controls valid: yes	(1979a)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay) Klim: 2 (reliable with restriction).	Test material (EC name): 2,3-epoxypropyl neodecanoate Form: liquid at room temp (purity not specified)	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100	Test concentrations: 1.0 – 1000 ug/plate. (met. act.: with and without)	Evaluation of results: **Positive without metabolic activation** Test results: positive for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested)	O.E.C.D. (2003g)
in vitro mammalian chromosome aberration test (chromosome aberration) As per A. P. Li and L.J. Loretz in "Genetic Toxicology" Chapter 6, Assays for Genetic Toxicology.CRC Press 1990, pp.119-141. Klim. 2 (reliable with restriction) Key study.	Test material (EC name): 2,3-epoxypropyl neodecanoate Form: liquid at room temp. (purity not specified).	Rat liver epithelial cell line RL1	Test concentrations: Final concentrations in treatment medium for separate experiments were: 0, 12.5, 25 and 50 ug/m1 or 0, 7.5, 15 and 30 ug/ml. (met. act.: with)	Evaluation of results: **Ambiguous with metabolic activation** (Rat liver epithelial cells have inherent metabolic capability.) Test results: ambiguous for primary culture, other: Rat liver derived RL1 cells. (strain/cell type: Rat liver epithelial RL1 cells.) ; met. act.: with ; cytotoxicity: yes	B. J. Dean, T.M. Brooks, G. Hodson—Walker, and G. Pook (1979b)
in vitro mammalian chromosome	Test material (EC name): 2,3-epoxypropyl	Chinese hamster Ovary (CHO)	Test concentrations: 4 hr treatment without S-9 metabolic activation:	Evaluation of results: **negative** (with and	S. Roy and M. Jois

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
aberration test (chromosome aberration) 20 hr treatment without S-9 metabolic activation: 0, 5, 10, 20, 25, 30, 40 ug/ml Positive control substance(s): mitomycin C Positive control substance(s): cyclophosphamide OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test) (in CHO cells.) Klim. 2 (reliable with restriction) key study	neodecanoate Form: Liquid at room temperature. (purity not specified)	(met. act.: with and without)	0, 5, 10, 20, 25, 30, 35, 40 ug/ml. 4 hr treatment with metabolic activation: 0, 1.0, 2.5, 5, 10, 15, 20, 25, 35 ug/ml	without rat liver S-9 metabolic activation.) Test results: negative for Chinese hamster Ovary (CHO)(all strains/cell types tested); met. act.: with and without ; cytotoxicity: yes ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	(2011)
yeast cytogenetic assay (genome mutation) equivalent or similar to OECD Guideline 481 (Genetic Toxicology: Saccharomyces cerevisiae, Mitotic Recombination Assay) Klim. 2 (reliable with restriction) experimental result	Test material (EC name): 2,3-epoxypropyl neodecanoate Form: Liquid at room temperature. (Purity not specified)	Saccharomyces cerevisiae (met. act.: with and without)	Test concentrations: 0.01, 0.1, 0.5, 1.0, and 5.0 mg/mL Positive control substance(s): EMS and 4NQO without S-9 metabolic activation preparation and Cyclophosphamide (CP) with S-9 metabolic activation.	Evaluation of results: **negative** (with and without S-9 metabolic activation.) Test results: **negative** for Saccharomyces cerevisiae(strain/cell type:) ; met. act.: with and without ; positive controls valid: yes	B. J. Dean, T.M. Brooks, G. Hodson—Walker, and G. Pook (1979b)
in vitro mammalian cell transformation assay (invitro cell transformation.) Styles, J. A. (1977). A method of detecting carcinogenic organic chemicals	Test material (EC name): 2,3-epoxypropyl neodecanoate Form: Liquid at room temperature (purity not specified).	Syrian hamster BHK cells primary culture, (met. act.:with)	Test concentrations: 0, 44, 87.5, 175 and 350 ug/mL Positive control substance(s): 7,12-dimethylbenzanthracene (at 25 and 50 ug/mL)	Evaluation of results: **negative** with metabolic activation Test results: **negative** for primary culture, other: Syrian hamster BHK cells(strain/cell	A. L. Meyer. (1981)

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
using mammalian cells in culture. Br. J. Cancer, 36, 558. Klim. 3 (unreliable) experimental result				type: BHK) ; met. act.: with ; cytotoxicity: yes ; negative controls valid: yes; positive controls valid: yes	

Gene mutations in bacteria:

EPDA induced gene-mutations in Ames/Salmonella tester strains TA 1535, TA 1537, TA98 and TA 100 with metabolic activation, but not without metabolic activation (Dawkes 1998) (OECD 471). Two other studies similar to OECD 471 also yielded positive results in the same strains. In one study EPDA was positive with metabolic activation, but not without (Dean et al. 1979a) and in the other study EPDA was only positive without metabolic activation (O.E.C.D SIDS 2003).

Gene mutations in yeast and mammalian cells:

A negative result was observed in a yeast cytogenetic assay (corresponding to OECD 481) both with and without metabolic activation (B. J. Dean et al., 1979)

No studies on gene mutations in mammalian cells were reported.

Chromosomal aberrations:

A negative result was obtained in a guideline *in vitro* mammalian chromosome aberration test using CHO cells (Roy et al., 2011)(OECD 473). Cells were tested for 4 hours with metabolic activation (at 1-35 µg/ml) as well as without metabolic activation (at 5-40 µg/ml). Cells were also treated for 20 hours without metabolic activation (at 5-40 µg/ml). Cells were harvested approximately 20 hours after the beginning of treatment.

A non-guideline *in vitro* mammalian chromosome aberration study using an epithelial—type cell line, designated RL1, derived from rat liver (with inherent metabolic capability) yielded an ambiguous result (Dean et al., 1979b). Final concentrations for separate experiments were 12.5-50 ug/ml or 7.5-30 ug/ml. In both cases, occasional chromatid aberrations were seen after 6 hours and 24 hours. Although the incidence of chromatid aberrations was very small, they occurred consistently in each of the experiments.

Table 8. Chromosome analysis of cultured rat liver (RL1) cells after 6 hours exposure to CARDURA E10 or methyl methanesulphonate (MMS).

CARDURA E10 (ex Pernis) µg/ml	No. of cultures	No. of cells analysed	Percentage cells showing				Percentage cells showing chromatid aberrations (3 + 4)
			1	2	3	4	
			Polyploidy	Chromatid gaps	Chromatid breaks	Exchange figures	
0	2	239	2.9	0	1.3	0	1.3
7.5	2	62	1.6	1.6	0	0	0
15	2	188	2.6	1.6	0.5	0	0.5
30	2	108	1.9	0	0	0.9	0.9
MMS 10 µg/ml	2	215	5.6	2.8	1.4	0	1.4

Table 9. Chromosome analysis of cultured rat liver (RL1) cells after 24 hours exposure to CARDURA E10 or methyl methanesulphonate (MMS).

CARDURA E10 (ex Pernis) µg/ml	No. of cultures	No. of cells analysed	Percentage cells showing					Percentage cells showing chromatid aberrations (3 + 5)
			1	2	3	4	5	
			Polyploidy	Chromatid gaps	Chromatid breaks	Acentric fragments	Exchange figures	
0	2	200	1	0.5	0	0	0	0
7.5	2	200	0.5	2.0	1.0	0	0	1.0
15	2	200	0.5	1.5	0.5	0	0	0.5
30	2	187	0	1.6	1.6	0	1.1	2.7
MMS 10 µg/ml	2	200	0	6.0	1.0	1.0	1.0	2.0

In vitro cell transformation Assay (genome mutation):

A negative result was obtained in an in vitro mammalian cell transformation assay from 1981 using Syrian hamster fibroblast kidney cells (BHK) with metabolic activation. The validity of the performance of the BHK cell line for rodent carcinogenicity is unknown (e.g. the number of rodent carcinogens and non carcinogens included in a validation exercise, its inter- and intra-laboratory variability and its sensitivity, specificity, positive and negative predictive values) as this study was conducted as a non-guidance study at the time. It is therefore not possible to draw any conclusion as to what the alleged negative result means in relation to the potential of EPDA for rodent carcinogenicity (Meyer, 1981).

10.8.1.1.2 *In vivo* data

Table 10. Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
Guideline 488 (somatic transgenic animal mutagenicity Assay) oral: gavage	EPDA in corn oil (purity approximate	Male mouse (Muta_Mouse CD2 lacZ80/HazfBR)	0. 250, 500 and 1000 mg/kg bw/d Positive control substance(s):	Evaluation of results: **positive** Test results: Genotoxicity: positive (Statistically significant, doserelated	Unpublished report (2012)

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
by intraperitoneal injection for all tissues sampled. Dose: Once per day on each of 42 consecutive days and sacrificed on Day 45 (42+3). Klim.1 (reliable without restriction)	ly 89%)		Ethylnitrosourea (ENU) at 100 mg/kg bw/d	increase of the mutant frequency in liver, kidney and bone marrow tissue.) Vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	
Guideline 488 (Germ cell transgenic animal mutagenicity assay). Oral: gavage Dose: Once per day on each of 28 consecutive days and sacrificed on day 78 (28+49). Klim. 2 (reliable with restrictions)	EPDA in corn oil (Purity was assumed as 100% for testing)	Mature sperm from male Muta™Mice (CD2-lacZ80/HazfBR strain)	1000 mg/kg bw/d for 28 days. Positive control substance; N-ethyl-N-nitrosourea (ENU)) at 150 mg/kg bw/d	Evaluation of results: **Equivocal.** Vehicle controls valid: yes; negative controls valid: not applicable; possitve controls valid: yes	Unpublished report (2019)
Alkaline elution detection of DNA single breaks. (DNA damage and/or repair) oral: gavage Petzold GL, Swenberg JA. Detection of DNA damage induced in vivo following exposure of rats to carcinogens. Cancer Res. 1978 Jun;38(6):1589-94. Klim. 3 (unreliable)	**2,3-epoxypropyl neodecanoate** Form: Liquid at room temp. (purity not specified).	rat (Wistar) male/female	Approximately 4850 mg/kg of body weight. (nominal conc.) Positive control substance(s): Methyl Methanesulphonate at 300 mg/kg of body weight in DMSO.	Negative vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Unpublished report (1981)

Method	Test substance	Organisms/ strain	Concentrations tested	Result	Reference
OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo) Administration: oral gavage Klim. 2 (reliable with restriction)	**2,3-epoxypropyl neodecanoate** Form: Liquid at room temperature. (purity not specified (100 % pr. Protocol).	Mate rat (Sprague-Dawley)	0. 500, 1000, 2000 mg/kg of body weight. (actual ingested (by oral gavage.)) Positive control substance(s): Dimethylnitrosamine at 35 mg/kg of body weitght	Genotoxicity: negative toxicity: yes ; vehicle controls valid: yes; negative controls valid: not applicable; positive controls valid: yes	Unpublished report (2011)

<u>*In vivo* Genotoxicity:</u>

Genotoxicity of EPDA was investigated in a non-guideline alkaline filter elution assay, which assesses single strand breaks and alkaline labile sites in DNA (unpublished report, 1981). Cells are layered onto a PVC membrane and washed with cold PBS and a lysing solution. Single strand damage is assessed as a reduction in single strand molecular weight (observed as an increase in rate of elution of radioactivity going through the filter). The rate of elution depends on the length of the single strands. EPDA did not not induce DNA damage *in vivo* in a rat alkaline elution study 6 hours after a single dose of 4850 mg/kg of body weight. Two males and two females were tested per group. Methyl methanesulphonate was administered in DMSO as a positive control. This is not a guideline study, group size was too small and only one dose was tested. No protease was used in the lysing solution, so it is possible that single strand breaks could still be adducted to proteins, which would mask a positive result.

An Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD 486) yielded a negative result (unpublished report, 2011). Four male rats (Harlan Sprague-Dawley) per dose and time interval were administered EPDA in corn oil by oral gavage at the final dose levels of 0, 500, 1000, and 2000 mg/kg of body weight. The duration of exposure was 2 to 4 hr and 12 to 16 hr per dose group. No significant increase in mean Net Nuclear Grain Counts (NNGC) or percent liver cells in DNA repair (UDS) was obtained. Dimethylnitrosamine at 35 mg/kg of body weight was used as a positive control. No significant increase in mean Net Nuclear Grain Counts (NNGC) or percent liver cells in DNA repair (UDS) was obtained.

<u>Gene mutations in vivo:</u>

In 2012 a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay was conducted in a MutaMouse (CD_2-lacZ80/HazfBR), whose DNA bearing cells each contain a transgenic lambda g10 vector with the bacterial lacZ gene (unpublished report, 2012). Exposure by oral gavage yielded a positive result in all somatic tissues tested. The study was conducted according to OECD 488 (2011) with GLP compliance and test substance concentration verification.

Seven male animals were tested per group. The animals were dosed with EPDA in corn oil once per day on each of 42 consecutive days (Days 1-42) and sacrificed on Day 45, i.e. 3 days after the final administration. A dose volume of up to 10 mL/kg of body was used. Dose volumes were based on individual body weight. Dose concentrations used were 0, 250, 500 and 1000 mg/kg bw/d. Tissues tested were liver, Kidney, Bone marrow and developing sperm cells from seminiferous tubules. The positive control used was Ethylnitrosourea (ENU) at 100 mg/kg bw/d by intraperitoneal injection for all tissues sampled. Statistical analyses were done using ANOVA, Dunnett's test and Levene's test. Plaque forming units (pfu) for each sample on any packaging

occation exceeded 30000 for the majority of samples. In a few cases pfus between 10000 and 30000 were accepted.

EPDA was shown to be a gene-mutagen in the liver, kidney and bone marrow of the MutaMouse demonstrating that the test substance is a systemic gene mutagen in mice by the oral route of exposure. In the liver at the high dose level (1000 mg/kg bw/d) the group mean mutant frequency was 3.1 -fold the mean concurrent vehicle control value. Although lower doses did not induce a significant increase in mutation frequency, an increase in group mean mutation frequency compared to the vehicle control was observed and a significant linear trend was also observed.

Table 11. Group summary - Liver

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10^{-6} ± SD)
1	Vehicle control	0	49.85 ± 18.91
2	CARDURA™E10P	250	68.07 ± 23.42
3	CARDURA™E10P	500	116.33 ± 51.26
4	CARDURA™E10P	1000	155.56 ± 139.89 *
5	ENU	100	561.13 ± 230.91 *** A,S DR***

* P≤0.05
*** P≤0.001
A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)
S two-sample t-test (Group 5 vs group 1)
DR Significant dose response test

For the kidney a statistically significant increase in mutant frequency was observed at all dose levels, a significant linear trend was also observed.

Table 12. Group summary - kidney

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10^{-6} ± SD)
1	Vehicle control	0	52.66 ± 22.19
2	CARDURA™E10P	250	104.81 ± 26.01 **
3	CARDURA™E10P	500	123.69 ± 17.45 ***
4	CARDURA™E10P	1000	114.00 ± 25.57 ***
5	ENU	100	739.23 ± 139.98 *** A,SR DR***

** P≤0.01
*** P≤0.001
A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)
S two-sample t-test (Group 5 vs group 1)
R Rank transformed data
DR Significant dose response test

For bone marrow statistically significant increases in mutation frequency were observed at 500 and 1000 mg/kg bw/d. No increase was observed for 250 mg/kg bw/d, however, a significant linear trend was observed.

Table 13. Group summary – bone marrow.

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10^{-6} ± SD)
1	Vehicle control	0	41.21 ± 9.44
2	CARDURA™E10P	250	43.86 ± 10.98
3	CARDURA™E10P	500	76.41 ± 14.89 **
4	CARDURA™E10P	1000	118.62 ± 19.80 ***
5	ENU	100	510.18 ± 346.39 *** AR,S DR***

** P≤0.01
*** P≤0.001
A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)
S two-sample t-test (Group 5 vs group 1)
R Rank transformed data
DR Significant dose response test

Mutation analysis of developing sperm cell from the seminiferous tubules showed no statistically significant increase in mutation frequency at any dose level and no significant linear trend was observed. All individual animals had mutation frequencies that were comparable with the concurrent vehicle control.

Table 14. Group summary – developing sperm cells from seminiferous tubules

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10^{-6} ± SD)
1	Vehicle control	0	27.83 ± 8.19
2	CARDURA™E10P	250	30.94 ± 12.26
3	CARDURA™E10P	500	30.29 ± 7.02
4	CARDURA™E10P	1000	26.13 ± 11.54
5	ENU	100	796.99 ± 165.10 *** A, SR

*** P≤0.001
A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)
S two-sample t-test (Group 5 vs group 1)
R Rank transformed data

In conclusion, the result of the TGR study shows that EPDA was found to be mutagenic in bone-marrow, kidney and liver tissue when exposed at up to 1000 mg/kg/ bw/d for 42 days and sampled 3 days later. The mutation frequency was not increased above the level of controls when germ cells from the seminiferous tubules were exposed and sampled under the same conditions.

TGR study in mature germ cells:

In a 2019 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (unpublished report, 2019) was conducted. EPDA was tested for its ability to induce gene mutation in the lacZ transgene in mature sperm from male Muta™Mice (CD2-lacZ80/HazfBR strain) in a 28 + 49 day regime. according to OECD Guideline 488. The result of this study was equivocal in males.

The TGR study included 4-7 male animals per group. The animals were dosed with EPDA in corn oil by oral gavage once per day on each of 28 consecutive days (Days 1-28) and sacrificed on Day 78, i.e. 50 days after the final administration. The study was conducted using a limit dose of 1000 mg/kg bw/d EPDA (CARDURA™ E10P), bilateral vas deferens and cauda epididymis were dissected from each animal and

mature sperm was retrieved according to established protocols. The positive control used was Ethylnitrosourea (ENU) at 150 mg/kg bw/d.

One group of 7 male MutaMice were exposed to 1000 mg/kg bw/d of EPDA in corn oil by oral gavage for 28 consecutive days. Animals were euthanized on day 78. Mice dosed in this study were 11-13 weeks old weighting 25-32 grams on the first day of dosing. Animal husbandry was conducted in accordance with the test guideline. No remarkable clinical observations were observed in either of the two groups.

Positive controls:

Tissues from 4 appropriate positive control treated animals (treated independently in the current study with 150 mg/kg bw/d N-ethyl-N-nitrosourea (ENU)) were used to provide DNA that were analyzed alongside the DNA from animals in this study, to confirm the correct functioning of the packaging reactions and platings in accordance with OECD TG 488. For two out of four animals in the positive control group pfus far below 200,000 was obtained (21,344 and 52,693 pfus respectively). The packaging reactions were in the range of 1-6.

Although two out of four animals had very low pfus in the ENU positive control group, the increase in MF was high and in the expected range, which indicate the assay has worked as expected.

Determination of mutant frequency:

Mutant frequency is determined by dividing the number of plaques/plasmids containing mutations in the transgene by the total number of plaques/plasmids recovered from the same DNA sample. No statistically significant increases in mutant frequency (MF) were observed in the mature sperm of treated male MutaMice. The MF of all individual animals were considered to be comparable with the concurrent vehicle control group and the MF of all animals fell within the laboratory's historical control data (41.07±42.06; based on 20 animals, range 13.82-188.17).

Table 15. Mutant frequency (MF) in mature sperm of treated male MutaMice.

Group	Treatment (dose)	Mutant frequency Group Mean MF (x 10^{-6})	Standard deviation	P-value
Vehicle control group (7 animals)	corn oil	46.16	14.91	-
Test group (7 animals)	EPDA (1000mg/kg bw/d)	53.18	9.32	0.1560 (NS)
Positive control (4 animals)	ENU (150 mg/kg bw/d)	339.86	48.85	<0.0001 (P≤0.001)

Statistical analyses:

According to the study director both ANOVA and a t-test were performed at the 5% level. The study director compared the vehicle control group to the treated group using a two-sample t-test. The t-test was interpreted with a one-sided risk for increasing response. The Levene's test for equality of variances between the groups was also performed and where this showed evidence of heterogeneity (P≤0.01), the data were rank-transformed prior to analysis. The positive control data were also compared to Group 1 as described above. Levene's test for equality of variances across the groups was also performed. In all cases there was no evidence of heterogeneity (P>0.01).

The Dossier Submitters repeated the statistical analysis excluding the 3 animals which fell below the 125,000 pfu limit described in the TG 488 guideline. When the one-sided t-test was repeated (using SigmaStat) without these 3 animals the increase in MF in the test group was statistically significant. Each group still included at least 5 animals (the minimum number of animals per group according to the test guideline). Data without the

3 low pfu animals passed the Normality test (Shapiro-Wilk test (P=0.109)) and the Equal variance test (P=0.621).

Table 16. **t-test recalculated by Dossier Submitters**

Group	Treatment (dose)	Mutant frequency Group Mean MF ($x\ 10^{-6}$)	Standard deviation	P-value
Vehicle control group (5 animals)	corn oil	39.59	11.02	-
Test group (6 animals)	EPDA (1000mg/kg bw/d)	52.76	10.14	P= 0.035 (P≤0.05)

The increase in MF in the test group compared to the vehicle group was very slight (1.33-fold), and even though the increase in MF is statistically significant, the biological relevance is unclear.

Furthermore, the fact that the data passed the normality and the equal variance test may be an indication that the two groups are not different from each other. Data from the TGR assay are generally not normally distributed (O'Brien 2014) and when there is a significant response, it is rare to have equal variance, which is why non parametric tests (or appropriate data transformation) are normally used in TGR statistical analyses.

As the total number of pfu's for some animals was below the limit recommended in the OECD test guideline, leading to a lower reliability on the results of the study. As a result, the Dossier Submitters has given the study a Klimisch 2 score (reliable with restrictions).

In conclusion, the result of the TGR study is equivocal due to the statistical significant response but unclear biological relevance of the very slight increase after removal of the animals with pfu´s below the limit recommended in the guideline.

10.8.1.2 Human data

Table 17. Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
There are no relevant human data				

10.8.2 Comparison with the CLP criteria

The CLP Criteria Guideline according with the CLP Regulation for classification of mutagenicity are divided into 3 different categories:

Classification as mutagenic, Category 1A (Muta1A; H340: May cause genetic defects) is based on evidence of a causal association between human exposure to the substance and heritable genetic damage.

Classification as mutagenic, Category 1B (Muta1B; H340: May cause genetic defects) is based on animal studies showing mutagenicity to germ cells either in assays on germ cells or by demonstrating mutagenic effects in somatic cells *in* vivo or *in vitro* as well as metabolic proof that the substances reaches the germ cells.

Classification as mutagenic, category 2 (Muta2; H341: Suspected of causing genetic defects) is based on animal studies showing mutagenity to germ cells either in assays on germ cells or by demonstrating mutagenic effects in somatic cells *in vivo* or *in vitro* as well as metabolic proof that the substances reaches the germ cells.

Classification in Category 2 may be based on positive results of a least one in vivo valid mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results.

In vitro results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens.

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

In general, mutations can be differentiated into gene mutations (e.g. point or frame shift mutation), chromosome mutations (structural chromosome changes) and genome mutations (loss or gain of whole chromosomes). Different mutagenicity tests may detect different types of mutations and genotoxic effects which have to be taken into account in the weight of evidence determination. For instance, a substance which only causes chromosome mutations may be negative in a test for detecting point mutations.

A complex data situation with positive and negative results might still lead to classification. This is because all tests detecting a certain type of mutation (e.g. point mutations) have been positive and all tests detecting chromosome mutations have been negative. Such circumstances clearly warrant classification although several tests have been negative which is plausible in this case.

Regarding the criteria from the CLP Guidance, a positive result for somatic or germinal mutagenicity in a test using intraperitoneal administration only shows that the tested substance has an intrinsic mutagenic property, and the fact that negative results are exhibited by other routes of dosage may be related to factors influencing the distribution/ metabolism of the substance which may be characteristic to the tested animal species.

At least one valid *in vivo* genotoxicity test using i.p. injection plus supportive *in vitro* data, classification is warranted. In cases where there are additional data from further *in vivo* tests with oral, dermal or inhalative substance application, a weight of evidence approach using expert judgement has to be applied in order to come to a decision.

For instance, it may be difficult to reach a decision on whether or not to classify in the case where there are positive *in vivo* data from at least one *in vivo* test using i.p.injection but (only) negative test data from (an) *in vivo* test(s) using oral, dermal, or inhalative application.

In such a case, it could be argued that mutagenicity/genotoxicity can only be shown at internal body substance concentrations which cannot be achieved using application routes other than intraperitoneal.

However, it also has to be taken into account that there is generally no threshold for mutagenicity unless there is specific proof for the existence of such a threshold as may be the case for aneugens. Thus, if mutagenicity/genotoxicity can only be demonstrated for the intraperitoneal route exclusively, then this may mean that the effect in the *in vivo* tests using application routes other than intraperitoneal may have been present, but it may not have been detected because it was below the detection limit of the oral, dermal, or inhalative test assays.

In summary, EPDA meets the requirements for classification as Muta 2; H341, under CLP based on the induced gene-mutation in the somatic tissues liver, kidney and bone marrow of the MutaMouse, in addition to a positive *in vitro* Ames test. Because mutagenicity in germ cells has not been demonstrated and there is no known causal association between human exposure to the substance and heritable genetic damage EPDA does not meet the requirements for a classification as mutagenic, Category 1A or 1B

10.8.3 Conclusion on classification and labelling for mutagenicity

EPDA should be classified as Muta 2. The corresponding hazard statement is H341: Suspected of causing genetic defects.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

DS proposed the classification of EPDA as Muta. 2, H341 based on gene mutations induced in liver, kidney and bone marrow of a transgenic mouse supported by positive results in several Ames tests.

Comments received during consultation

During consultation, one MSCA supported the classification proposed by the DS although questioned the biological relevance of the results obtained with the transgenic mouse based on statistical gaps, marginal increase in the mean mutation frequency and lack of differences with historical control data of the performing facility.

The second MSCA considered that this was a borderline case between Muta. 2 and no classification with results in favour and against classification. This MSCA also asked clarification about route of exposure in one transgenic rodent assay and whether there are indications that germ cells were reached in transgenic rodent studies. The DS disagreed with the consideration of borderline case since there are consistent findings observed for induction of gene mutations in *in vitro* studies, and additionally *in vivo* positive results in various somatic tissues are available. The DS clarified that animals were dosed by gavage in the 2012 transgenic rodent study and there are no indications of whether the germ cells were reached in transgenic rodent (TGR) studies.

Assessment and comparison with the classification criteria

The results of the mutagenicity/genotoxicity *in vitro* studies with EPDA are summarised in Table below. The database contains bacterial reverse mutation assays, a gene mutation assay in yeast, chromosomal aberration tests and an *in vitro* cell transformation assay.

EPDA yielded positive results with metabolic activation in up to 4 different bacterial strains of Salmonella and in 2 up to Escherichia strains in two different Ames tests; while a third Ames test yielded positive results in the four Salmonella strains but without metabolic activation.

Other *in vitro* tests yielded negative or inconclusive results. Specifically, in a yeast gene mutation assay (no studies on gene mutations in mammals were found in the CLH report), in a chromosomal aberration tests in Chinese hamster Ovary (CHO) cells and in epithelial-type liver cells of a transgenic mouse. In addition, a negative result in an unreliable mammalian cell transformation assay with Syrian hamster fibroblast kidney cells was noted in the CLH report.

***Table*:** *Summary of mutagenicity/genotoxicity in vitro studies with EPDA*

Method	Tested concentrations	Results	Reference
Bacterial reverse mutation assay OECD TG 471 Klimisch score: 1 2,3-epoxypropyl neodecanoate (purity not specified) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100	Test concentrations: 1.6, 8, 40, 200, 1000, and 5000 µg/plate for the 1st mutation study 125, 250, 500, 1000, 2000, and 5000 µg/plate for the 2nd mutation study Both trials conducted with and without rat liver S9 metabolic activation Positive controls: Yes	Cytotoxicity between 1000 and 5000 µg/plate Vehicle controls valid: Yes Negative controls valid: Yes Positive controls valid: Yes **Positive in all strains with metabolic activation**	Dawkes, 1998
Bacterial reverse mutation assay Equivalent or similar to OECD TG 471 Klimisch score: 2 2,3-epoxypropyl neodecanoate (containing 0.096% epichlorohydrin) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* strains WP2 and WP2 uvrA	Test concentrations: 0, 0.2, 2, 500 and 2000 µg/plate With and without rat liver S9 metabolic activation Positive control substances: Yes	Positive controls valid: Yes **Positive in all strains with metabolic activation**	Dean, Brooks, Hodson-Walker, and Pook, 1979a
Bacterial reverse mutation assay Equivalent or similar to OECD TG 471 Klimisch score: 2 2,3-epoxypropyl neodecanoate (purity not specified)	Test concentrations: 1.0-1000 µg/plate With and without rat liver S9 metabolic activation	**Positive in all strains without metabolic activation**	OECD SIDS (2003)

<table>
<tr><td>S. typhimurium TA 1535, TA 1537, TA 98 and TA 100</td><td></td><td></td><td></td></tr>
<tr><td>In vitro mammalian chromosome aberration test

As per A. P. Li and L.J. Loretz in "Genetic Toxicology" Chapter 6, Assays for Genetic Toxicology. CRC Press 1990, pp.119-141.

Klimisch score: 2

2,3-epoxypropyl neodecanoate (purity not specified)

Rat liver epithelial cell line RL1</td><td>Final concentrations: 0, 12.5, 25 and 50 µg/mL or 0, 7.5, 15 and 30 µg/mL

With and without rat liver S9 metabolic activation</td><td>Cytotoxicity with metabolic activation: Yes

Ambiguous with metabolic activation

Chromosome analysis of cultured RL1 rat liver cells
<table><tr><td></td><td colspan="2">% chromatid aberrations</td></tr><tr><td>[µg/mL]</td><td>6 h</td><td>24 h</td></tr><tr><td>0</td><td>1.3</td><td>0</td></tr><tr><td>7.5</td><td>0</td><td>1.0</td></tr><tr><td>15</td><td>0.5</td><td>0.5</td></tr><tr><td>30</td><td>0.9</td><td>2.7</td></tr><tr><td>Pos. control</td><td>1.4</td><td>2.0</td></tr></table></td><td>Dean, Brooks, Hodson-Walker, and Pook, 1979b</td></tr>
<tr><td>In vitro mammalian chromosome aberration test

OECD TG 473

Klimisch score: 2

2,3-epoxypropyl neodecanoate (purity not specified)

Chinese hamster Ovary (CHO)</td><td>20 h treatment without S-9 metabolic activation: 0, 5, 10, 20, 25, 30, 40 µg/mL

4 h treatment without S-9 metabolic activation: 0, 5, 10, 20, 25, 30, 40 µg/mL

4 h treatment with S-9 metabolic activation: 0, 1.0, 2.5, 5, 10, 20, 25, 35 µg/mL

Positive controls: mitomycin C and cyclophosphamide</td><td>Cytotoxicity: Yes

Vehicle controls valid: Yes

Positive controls valid: Yes

Negative with and without metabolic activation</td><td>Roy and Jois, 2011</td></tr>
<tr><td>Yeast cytogenetic assay (genome mutation)

Equivalent or similar to OECD TG 481

Klimisch score: 2</td><td>Test concentrations: 0.01, 0.1, 0.5, 1.0, and 5.0 mg/mL

Positive controls: EMS and 4NQO (without S-9 metabolic activation) and cyclophosphamide (with S-9 metabolic activation)</td><td>Positive controls valid: Yes

Negative (with and without S-9 metabolic activation)</td><td>Dean, Brooks, Hodson-Walker, and Pook, 1979b</td></tr>
</table>

2,3-epoxypropyl neodecanoate (Purity not specified) *S. cerevisiae*			
In vitro mammalian cell transformation assay Klimisch score: 3 2,3-epoxypropyl neodecanoate (purity not specified) Syrian hamster baby hamster kidney (BHK) cells	Test concentrations: 0, 44, 87.5, 175 and 350 µg/mL Positive controls: 7,12-dimethylbenzanthracene Only with rat liver S9 metabolic activation	Cytotoxicity: Yes Negative controls valid: Yes Positive controls valid: Yes **Negative with metabolic activation**	Meyer, 1981

RAC highlights that the yeast cytogenic assay seems to use EPDA concentrations (0.01-5 mg/mL) apparently higher than the solubility limit in water (70 mg/L). No information about vehicle is provided in the information available to RAC. Thus, given the gaps, RAC will put less weight to this study in the final proposal.

The results of the mutagenicity/genotoxicity in *in vivo* studies with EPDA are summarised in Table below. The database contains transgenic rodent somatic and germ cell gene mutation assays, a test for detection of DNA damage single breaks and an unscheduled DNA synthesis in liver cells.

A guideline unscheduled DNA synthesis test with liver rat yielded a negative result as well as a non-guideline alkaline filter elution assay, which assess single strand breaks. However, the transgenic rodent germ cell gene mutation assay yielded equivocal results while the results in all somatic cells (liver, kidney and bone marrow) were positive.

Table: *Summary of mutagenicity/genotoxicity in vivo studies with EPDA*

Method	Tested concentrations	Results	Reference
OECD TG 488 GLP: Yes Somatic and germ cell transgenic animal mutagenicity assay Klimisch score: 1	Dose: Once per day on each of 42 consecutive days and sacrificed on day 45 (42+3) 0. 250, 500 and 1000 mg/kg bw/day Positive control: ethylnitrosourea (100 mg/kg bw/day) by intraperitoneal injection	Vehicle controls valid: Yes Positive controls valid: Yes **Positive** (statistically significant, dose-related increase of the mutant frequency in liver,	Unpublished report, 2012

EPDA in corn oil (purity approximately 89%) Oral gavage 7 male mouse (CD2 lacZ80/HazfBR strain)/group		kidney and bone marrow tissue) **Negative** in developing sperm cells from seminiferous tubules	
OECD TG 488 Germ cell transgenic animal mutagenicity assay Klimisch score: 2 EPDA in corn oil (purity was assumed as 100% for testing) Oral gavage Mature sperm from CD2-lacZ80/HazfBR strain	7 males: 1000 mg/kg bw/day for 28 days in corn oil during 28 days (euthanized on day 78) 4 males: Positive control: N-ethyl-N-nitrosourea at 150 mg/kg bw/day 7 males: vehicle control	Vehicle controls valid: Yes Positive controls valid: Yes **Equivocal**	Unpublished report, 2019
Alkaline elution detection of DNA single breaks Klimisch score: 3 2,3-epoxypropyl neodecanoate (purity not specified) Wistar rats 2 animals/sex	Approximately 4850 mg/kg bw Positive control: Methyl methanesulphonate at 300 mg/kg bw	Vehicle controls valid: Yes Positive controls valid: Yes No protease was used in the lysing solution, so it is possible that single strand breaks could be adducted to proteins, which would mask a positive result **Negative**	Unpublished report, 1981

OECD TG 486 Unscheduled DNA Synthesis (UDS) Klimisch score: 3 2,3-epoxypropyl neodecanoate (purity not specified) Oral gavage 4 male Sprague-Dawley rats	0, 500, 1000, 2000 mg/kg bw in corn oil Positive control: dimethylnitrosamine at 35 mg/kg bw	Vehicle controls valid: Yes Positive controls valid: Yes No significant increase in mean net nuclear grain counts or % liver cells in DNA repair **Negative**	Unpublished report, 2011

Somatic cell mutagenicity assay in transgenic rodent

In the experimental conditions shown in Table above (Unpublished report, 2012), EPDA was shown to be a gene-mutagen in the liver, kidney and bone marrow, but not in developing sperm cells from seminiferous tubules. In the liver, at the highest dose level the group mutant frequency was 3.1-fold the mean of the concurrent vehicle control value (Table below). For the kidney, a statistically significant increase in mutant frequency was observed at all dose levels (Table below). For bone marrow, statistically significant increases in mutation frequency were observed at 500 and 1000 mg/kg bw/day. No statistically significant mutations were noted in developing sperm cells from seminiferous tubules.

Table: *Mutant frequency in the somatic and germ cell transgenic animal mutagenicity assay*. EPDA was dosed by intraperitoneal injection. Positive control was 100 mg/kg bw/day ethylnitrosourea (by intraperitoneal injection) * = p<0.05; ** = p<0.01; *** = p<0.001

	Mutant frequency (mean±SD) x 10^6			
Treatment	**Liver**	**Kidney**	**Bone marrow**	**Developing sperm cells**
Vehicle	49.85±18.91	52.66±22.19	41.21±9.44	27.83±8.19
250 mg/kg bw/day	68.07±23.42	104.81±26.01**	43.86±10.98	30.94±12.26
500 mg/kg bw/day	116.33±51.26	123.69±17.45***	76.41±14.89**	30.29±7.02
1000 mg/kg bw/day	155.56±139.89*	114.00±25.57***	118.62±19.80***	26.13±11.54
Positive control	561.13±230.91***	739.23±139.98***	510.18±346.39***	796.99±165.10***

Germ cell mutagenicity assay in transgenic rodent

Table above describes the experimental conditions of a germ cell gene mutation assay performed with transgenic rodent (Unpublished report (2019)) that yielded an equivocal result.

In the first statistical analysis, the mutant frequency of all individual animals was considered comparable to concurrent vehicle control group and fell within the historical control data of the performing facility (Table below). However, the DS repeated the statistical analysis excluding the three animals, which fell below the 125 000-plaque forming units limit described in the OECD TG 488. In this new statistical assessment, each group still included at least 5 animals (the minimum number of animals/group according to the test guideline) and the increase in mutant frequency in the test group was statistically higher than in the vehicle group (second Table below).

Table: *Mutant frequency in mature sperm of treated mutant mice.*
It is shown original report assessment.

Group	Treatment	Mutant frequency (mean±SD) x 10^6	p
Control (7 animals)	Corn oil	46.16±14.91	-
Test group (7 animals)	1000 mg/kg bw/day EPDA	53.18±9.32	0.15
Positive control (7 animals)	150 mg/kg bw/day N-ethyl-N-nitrosourea	339.86±48.85	< 0.001

Table: *Mutant frequency in mature sperm of treated mutant mice*
It is shown DS calculation after removing 2 animals with plaque forming units below the threshold determined in the OECD Guideline.

Group	Treatment	Mutant frequency (mean±SD) x 10^6	p
Control (5 animals)	Corn oil	39.59±11.02	-
Test group (6 animals)	1000 mg/kg bw/day EPDA	52.76±10.14	0.035

In conclusion, the results of this study are considered by RAC as equivocal due to the statistically significant response but unclear biological relevance of the very slight increase (1.3-times) after removal animals with plaque forming units below the limit recommended by the guideline.

Comparison with the criteria

The CLH report does not contain human data and therefore the classification as Muta. 1A is not warranted.

The CLP regulation considers that the classification as Muta. 1B is based on animal studies showing mutagenicity to germ cells either in assays on germ cells or by demonstrating mutagenic effects in somatic cells as well as metabolic proof that substance reaches germ cells. Table above on *in vivo* studies shows negative results in germ cells and positive results in somatic cells. Moreover, there are no toxicokinetic evidence supporting the possibility that EPDA could reach germ cells. Thus, the classification as Muta. 1B is not warranted.

Classification as Muta. 2 is based on animal studies showing mutagenicity to somatic cell mutagenicity tests *in vivo* in mammals; or other *in vivo* somatic cell genotoxicity tests, which are supported by positive results from *in vitro* mutagenicity assays. Table above on *in vivo* studies shows that EPDA was able to induce mutagenicity *in vivo* in liver, kidney and bone marrow after intraperitoneal dosage. This observation is supported by positive results in bacterial reverse mutation assays (Table above on *in vitro* studies).

Moreover, RAC notes that the epoxide group of EPDA represents a structural alert for genotoxicitywhich also supports the necessity of classification.

Overall, **RAC supports the DS's proposal for classification of EPDA as Muta. 2 with the hazard statement H341 (suspected of causing genetic defects).**

10.9 Carcinogenicity

Hazard class not assessed in this dossier

10.10 Reproductive toxicity

Hazard class not assessed in this dossier

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier

10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier

10.13 Aspiration hazard

Hazard class not assessed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Hazard class not assessed in this dossier

13 ADDITIONAL LABELLING

The substance fulfils the criteria for supplemental hazard in Annex II point 2.8, that aims at protecting already sensitised individuals. An elicitation concentration limit 0.0001%, i.e. one tenth of the specific concentration limit of 0.001%, will apply to EPDA (cf. CLP Annex I, table 3.4.6).

The supplemental hazard information: EUH208 – 'Contains 2,3-epoxypropyl neodecanoate (EPDA). May produce an allergic reaction' should be included in the label on the packaging of mixtures not classified as sensitising but containing EPDA in a concentration above or equal to 0.0001%.

Specific labelling requirement aiming at protecting already sensitised individuals are set in the CLP criteria. In accordance with CLP Annex I table 3.4.6 and its corresponding note, the elicitation limit of 0.0001% (one tenth of the specific concentration limit set above under point 7.9 above) will apply for EPDA. The supplemental labelling of Annex II point 2.8, “EUH208 – 'Contains 2,3-epoxypropyl neodecanoate (EPDA). May produce an allergic reaction' “ will apply for mixtures containing EPDA at or above i.e. 0.0001% , when not leading to classification as a skin sensitiser.

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15 ANNEXES

Not relevant