Committee for Risk Assessment

RAC

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

Adopted

18 March 2022

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18 March 2022

CLH-O-0000007104-83-01/F

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

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

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

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

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

The proposal was submitted by **Denmark** and received by RAC on **5 May 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **7 June 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **6 August 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2022** by **consensus**.

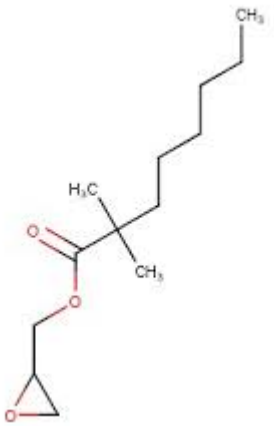
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2,3-epoxypropyl neodecanoate	247-979-2	26761-45-5	Muta. 2 Skin Sens. 1A	H341 H317	GHS08 GHS07 Wng	H341 H317		Skin Sens. 1A; H317: C ≥ 0,001%	
RAC opinion	TBD	2,3-epoxypropyl neodecanoate	247-979-2	26761-45-5	Muta. 2 Skin Sens. 1A	H341 H317	GHS08 GHS07 Wng	H341 H317		Skin Sens. 1A; H317: C ≥ 0,001%	
Resulting Annex VI entry if agreed by COM	TBD	2,3-epoxypropyl neodecanoate	247-979-2	26761-45-5	Muta. 2 Skin Sens. 1A	H341 H317	GHS08 GHS07 Wng	H341 H317		Skin Sens. 1A; H317: C ≥ 0,001%	

GROUNDS FOR ADOPTION OF THE OPINION

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. The 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. The 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.

HUMAN HEALTH HAZARD EVALUATION

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 tests (GPMT), two of them warranting category 1A for EPDA and the other two not contradicting this classification. Moreover, two of these GPMT showed an extreme potency for EPDA; which allowed the DS to 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: 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	Unpublished report, 1998 Klimisch score: 2

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 the DS due to inconsistencies). These studies were summarised in Table below. According to these data 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).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The 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	Test concentrations: 0, 0.2, 2, 500 and 2000 µg/plate With and without rat liver S9 metabolic activation	Positive controls valid: Yes **Positive in all strains with metabolic activation**	Dean, Brooks, Hodson-Walker, and Pook, 1979a

<table>
<tr><td>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</td><td>Positive control substances: Yes</td><td></td><td></td></tr>
<tr><td>Bacterial reverse mutation assay

Equivalent or similar to OECD TG 471

Klimisch score: 2

2,3- epoxypropyl neodecanoate (purity not specified)

S. typhimurium TA 1535, TA 1537, TA 98 and TA 100</td><td>Test concentrations: 1.0-1000 µg/plate

With and without rat liver S9 metabolic activation</td><td>Positive in all strains without metabolic activation</td><td>OECD SIDS (2003)</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

<table>
<tr><td colspan="3">Chromosome analysis of cultured RL1 rat liver cells</td></tr>
<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</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,</td><td>Cytotoxicity: Yes

Vehicle controls valid: Yes

Positive controls valid: Yes</td><td>Roy and Jois, 2011</td></tr>
</table>

Klimisch score: 2 2,3- epoxypropyl neodecanoate (purity not specified) Chinese hamster Ovary (CHO)	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	**Negative with and without metabolic activation**	
Yeast cytogenetic assay (genome mutation) Equivalent or similar to OECD TG 481 Klimisch score: 2 2,3- epoxypropyl neodecanoate (Purity not specified) *S. cerevisiae*	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)	Positive controls valid: Yes **Negative (with and without S-9 metabolic activation)**	Dean, Brooks, Hodson-Walker, and Pook, 1979b
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 EPDA in corn oil (purity approximately 89%) Oral gavage 7 male mouse (CD2 lacZ80/HazfBR strain)/group	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, kidney and bone marrow tissue) **Negative** in developing sperm cells from seminiferous tubules	Unpublished report, 2012
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 the 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 genotoxicity which 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).**

ANNEXES:

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

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