Helsinki, 1 March 2017

Substance name: 2,3-epoxypropyl neodecanoate
EC number: 247-979-2
CAS number: 26761-45-5
Date of Latest submission(s) considered: June 2016
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[1] of the registered substance 2,3-epoxypropyl neodecanoate

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on the registered substance, 2,3-epoxypropyl neodecanoate (abbreviated EPDA in the following):

Human health endpoint Mutagenicity:
Transgenic rodent somatic and germ cell gene mutation assay (OECD 488) in mice, oral route. Dosing shall be done by oral gavage daily in an appropriate freshly prepared vehicle solution/formulation for 28 days at 1000 mg/kg/day and sampling shall be done 3 days and 7 weeks after end of exposure. Germ cells from vas deferens shall be sampled and analysed. The evaluating MSCA must have access to the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA.

You shall provide an update of the registration dossier(s) containing the requested information, including the robust study summary and the full study report, with all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA, and where relevant, an update of the Chemical Safety Report by **10 December 2018**. The deadline takes into account the time that you may need to agree on which of the registrant(s) will perform the required test.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

[1] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

2. Who performs the testing

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all Registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

3. Appeal

You can appeal this decision to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals

Authorised[2] by Leena Ylä-Mononen, Director of Evaluation

[2] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on 2,3-epoxypropyl neodecanoate (EPDA) and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (MSCA) to complete the evaluation of whether the substance constitutes a risk to human health.

The evaluating MSCA will subsequently review, in the follow-up process, the information submitted by you and evaluate if further information should be requested in order to clarify the concerns for skin sensitisation, mutagenicity and carcinogenicity.

ENDPOINT Mutagenicity

The Concern(s) Identified

According to information from the registration dossier 2,3-epoxypropyl neodecanoate (EPDA) is mutagenic *in vivo* in the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay OECD (TG 488) in bone marrow, kidney and liver tissue when exposed at up to 1000 mg/kg/day for 42 days and sampled 3 days later ([redacted] 2012). 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. Based on these results the substance is self-classified as MUTA 2.

The susceptibility of male germ cells to chemical mutagens is highly dependent on the stage of development that the germ cell is in. The most susceptible stage varies from chemical to chemical. Therefore, it is important that all cell stages have been sufficiently exposed and that samples are collected, which correspond to these stages of germ cell development.

In order to conclude on germ cell mutagenicity the OECD 488 guideline recommends sampling cells from the vas deferens exposed for 28 days and sampled after *both* 3 days and 7 weeks. Alternatively, *both* the seminiferous tubules and vas deferens should be sampled 3 days after a 28 day exposure. This last strategy will provide some coverage of cells exposed across the majority of phases of germ cell development. Cells sampled from the seminiferous tubules are a mixed population of germ cells in different stages of development as well as somatic cells. It is therefore possible that a mutagenic effect, which occurs in a subgroup of germ cells in a susceptible developmental stage will be masked by this mixed population and hence not be statistically significant. This is not the case for mature germ cells sampled from the vas deferens, which will all be at the same stage of development.

In the [redacted] (2012) study the mutation frequency was only investigated in cells from the seminiferous tubules. Samples from the vas deferens were also collected, but were never analysed.

The available information on germ cell mutagenicity is insufficient to conclude on classification and the evaluating MSCA is therefore concerned that EPDA may be a germ cell mutagen MUTA 1B. If this is the case the current risk management measures are insufficient, leading to a risk to the user of EPDA as a substance and in mixtures, both in the working environment and for the general public. A review of the available studies on mutagenicity has been performed under this substance evaluation. Unless otherwise stated all evaluated studies were part of the registration dossier.

Gene mutations in bacteria

EPDA induced gene-mutations in Ames/Salmonella tester strains TA 1535,TA 1537, TA98, TA 100 with metabolic activation, but not without (OECD 471), (evaluating MSCA: Reliable (Klim. 1)). 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 (evaluating MSCA: Reliable with restrictions (Klim. 2)) and in the other study EPDA was only positive without metabolic activation (evaluating MSCA: Reliable with restrictions (Klim. 2)).

Gene mutations in yeast and mammalian cells

A negative result was observed in a yeast cytogenetic assay (OECD 481; both with and without metabolic activation. However, this study lacked a positive control and details of the study were not well-described and it is therefore not reliable (evaluating MSCA: Unreliable (Klim. 3)).

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 (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 (evaluating MSCA: Reliable with restrictions (Klim 2)).

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. 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 (evaluating MSCA: Reliable with restrictions (Klim 2)).

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 test method used in this test was from before the SHE and Bhas cell transformation assays were drafted for consideration as OECD Guidance Documents. Hence, the validity of the performance of the BHK cell line for rodent carcinogenicity is unknown (e.g. as

regards 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). Further, cultures were exposed to 7, 12—dimethyl—benzanthracene to serve as positive controls. It is not possible to draw any conclusion as to what the alleged negative result means in relation to the potential of EPDA for rodent carcinogenicity (evaluating MSCA: Not reliable (Klim. 3)).

In vivo Genotoxicity

Genotoxicity of EPDA was investigated with an alkaline filter elution assay, which assesses single strand breaks and alkaline labile sites in DNA. 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 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 (evaluating MSCA: Klim. 3, not reliable).

An Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD 486) yielded a negative result. 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 (evaluating MSCA: Reliable with restrictions (Klim. 2)).

Gene mutations *in vivo*

A Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay was conducted in a MutaMouse (CD_2-lacZ80/HazfBR). Exposure by oral gavage yielded a positive result in all somatic tissues tested. The study was conducted according to OECD 488 (2011) ([redacted] 2012) (evaluating MSCA: Reliable (Klim 1)).

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. The exposure and sampling time for this study was not justified in the study report. Dose concentrations used were 0. 250, 500 and 1000 mg/Kg of body weight per day. Tissues tested were liver, kidney, bone marrow and developing sperm cells from seminiferous tubules. The positive control used was Ethylnitrosourea (ENU). 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/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. For the kidney a statistically significant increase in mutant frequency was observed at all dose levels, a significant linear trend was also observed. For bone marrow, statistically significant increases in mutation frequency were observed at 500 and 1000 mg/kg/day. No increase was observed for 250 mg/kg/day, however, a significant linear trend was observed.

Germ cell mutagenicity *in vivo*

Germ cell mutagenicity was assessed by sampling cells in the seminiferous tubules in the TGR assay described above using an exposure time of 42 days and sampling 3 days later ([redacted] 2012). 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.

Vas deferens tissue containing mature sperm cells was collected in the study, but was not analysed but rather only stored.

It is known from the scientific literature that different stages of male germ cells differ in their susceptibility to mutagens. This susceptibility is highly dependent on the stage of development that the germ cell is in as well as on which chemical agent is being tested (c.f. e.g. Wyrobek *et al.* 2007).

Therefore, it is important that all cell stages have been sufficiently exposed and that samples are collected, which correspond to these stages of germ cell development. In order to conclude on germ cell mutagenicity the OECD 488 guideline recommends sampling cells from the vas deferens exposed for 28 days and sampled after *both* 3 days and 7 weeks. Alternatively, *both* the seminiferous tubules and vas deferens should be sampled 3 days after a 28 day exposure. This last strategy will provide some coverage of cells exposed across the majority of phases of germ cell development and may be useful for detecting some germ cell mutagens.

Cells sampled from the seminiferous tubules are a mixed population of germ cells in different stages of development as well as somatic cells. It is therefore possible that a mutagenic effect, which occurs in a subgroup of germ cells in a susceptible developmental stage will be masked by this mixed population and hence not be statistically significant. The sensitivity of the test applied to cells retrieved from testicular tissue (i.e. cells from a variety of spermatogenic phases) has not been rigorously tested (Yauk et al 2015).

Cells from the seminiferous tubules which have been exposed for 42 days and sampled 3 days later, as is the case for the [redacted] 2012 study, will have been exposed through the (pre-meiotic, mitotic) stages of stem cells and spermatogonia; as spermatocytes (during meiosis); and as spermatids and immature stages of sperm (post-meiotic). There are indications in the literature that sampling seminiferous tubules may result in an underrepresentation of cells, which were exposed at the spermatogonial stage when cells differentiate and divide mitotically and DNA repair is still active, which may increase the likelihood of gene mutations resulting from erroneous DNA repair during DNA synthesis (Yauk et al 2015).

Germ cells exposed for 42 days and sampled at day 45 as mature sperm cells from the vas deferens will not have been exposed during the stem cell stage, but through all other stages of their development. The exposure will have started 1 week prior to meiosis and exposure will have taken place for 1 week during the spermatogonial stage. Cells will also have been exposed during meiosis and through all later stages of development.

Summary for mutagenicity

EPDA is clearly positive for gene mutations in somatic tissues *in vivo* in all tissues tested (liver, kidney, bone marrow). There is sufficient evidence to classify EPDA in category MUTA 2 according to CLP. No evidence of an increased mutation frequency was observed in the developing sperm cells in this study. However, the potential for EPDA to cause mutations was not assessed in all stages of spermatogenesis, but only in developing sperm cells sampled from a mixed cell population in the seminiferous tubules. Consequently, germ cell mutagenicity cannot be ruled out and there is a residual concern for gene mutagenicity in germ cells.

Further testing is needed in order to clarify if EPDA should be classified as MUTA cat 1B. In order to conclude on germ cell mutagenicity, germ cells should be exposed and sampled according to the guideline: Exposed for 28 days and sampled at both 3 days and 7 weeks later. If the tissue from vas deferens, which was collected in the TGR study has not been destroyed, you are requested to analyse this tissue instead, which will provide the needed evidence to be able to conclude on the concern for germ cell mutagenicity.

Why new information is needed

There is a potential risk of human health effects due to the mutagenic properties of EPDA. The available data from the TGR study are unable to address the residual concerns about the potential of EPDA and/or its reactive metabolites to induce heritable gene mutations in germ cells (i.e. whether EPDA should be classified MUTA Cat 1B according to the CLP regulation). Hence, the proposed classification as MUTA Cat 2 may not be sufficient to ensure safe use. It is noted that a harmonized MUTA Cat 1B classification in accordance with the CLP Regulation would elicit various downstream risk management measures according to existing EU legislation, which would limit the exposure to EPDA and also make it possible for an EU CA to propose to include EPDA on the Candidate List of REACH as an initial step in the Authorisation REACH procedures.

Considerations on the test method and testing strategy

A residual concern remains for germ cell mutagenicity. This concern can be clarified by conducting a limited Transgenic rodent somatic and germ cell gene mutation assay (OECD 488) in mice, which only samples and investigates germ cells from vas deferens and doses daily for 28 days at 1000 mg/kg/day and samples at 3 days and 7 weeks after the end of exposure. A TGR assay has already been conducted ([redacted] 2012) and the relevant tissue has been collected, which would enable the test facility, which conducted the original study to analyse the DNA from mature sperm cells using the same study protocol. However, based on your comments, the collected tissue is no longer available at the contract laboratory which conducted the TGR assay in 2012.

Therefore, a new limited TGR assay shall be conducted according to the study protocol used by [redacted] 2012: Using the same methods, test system, test article formulation, vehicle, negative control, positive control, and exposure route. However, the duration of exposure should be 28 days and sampling of vas deferens should be done 3 days and 7 weeks after the end of exposure. As EPDA is a reactive substance it may react in the administration formulation and hence a freshly prepared testing dose in an appropriate vehicle shall be used.

Alternative approaches and Proportionality of the request

A new limited TGR assay will address the residual concern for germ cell mutagenicity and enable to conclude on the endpoint of mutagenicity by testing only one high dose sampled at 2 different time points as well as positive and negative controls. This test will use a total of 28 animals (7 animals per group).

It is noted that, if the vas deferens tissue samples would have been available for reliable analysis, such analysis could have been performed without conducting a new animal study or sacrificing any more animals. Unfortunately, this is not the case and the only way to conclude on the endpoint of mutagenicity is by performing a new limited TGR assay.

Considerations of Registrant(s)' comments

The evaluating MSCA notes that the samples from the vas deferens collected during the previously performed Transgenic rodent somatic and germ cell gene mutation assay (OECD 488) ([redacted] 2012) unfortunately are no longer available from the contract laboratory. Further testing in germ cells is therefore needed in order to clarify if EPDA should be classified as MUTA cat 1B.

The evaluating MSCA acknowledges your response agreeing to perform the limited Transgenic rodent somatic and germ cell gene mutation assay (OECD 488) in mice sampling only germ cells from vas deferens and dosing daily for 28 days at 1000 mg/kg/day and sampling 3 days and 7 weeks after end of exposure as requested in the draft decision.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the Analysis of mature sperm cells in the Transgenic rodent somatic and germ cell gene mutation assay (OECD 488) in mice sampling only germ cells from vas deferens and dosing daily for 28 days at 1000 mg/kg/day and sampling after 3 days and 7 weeks.

The requested limited TGR OECD 488 study will be conducted using the same method, test system, test article formulation, vehicle, negative and positive control and exposure route as previously used in [redacted] 2012.

You are reminded that the requested exposure time (28 days) and sampling periods (both 3 days and 7 weeks later) in the requested limited TGR OECD 488 study are different from those used in the previous study (OECD 488 [redacted] 2012, which

sampled 3 days after a 42 day exposure period.

To ensure a maximal exposure to unreacted EPDA, preparations of test formulations shall be freshly made daily in the new study, no later than 20 minutes before administration of each dosage. Analyses of homogeneity and stability of the test formulations shall be performed, and this shall be documented in the study report. The duration of the gavage procedure for each group shall also be documented in the study report.

The evaluating MSCA must have access to the full study report from the requested study including all relevant details of the study. Access to such detailed test report information is in the experience of the evaluating MSCA often needed to ensure that a clear conclusion regarding the result of the study can be drawn.

References

[redacted] (included in the registration dossier).

Wyrobek et al (2007). 'Assessing human germ-cell mutagenesis in the Postgenome Era: a celebration of the legacy of William Lawson (Bill) Russell'. Environ Mol Mutagen.;48(2):71-95.

Yauk CL, et al (2015). 'Approaches for identifying germ cell mutagens: Report of the 2013 IWGT workshop on germ cell assays'. Mutat Res Genet Toxicol Environ Mutagen;783:36-54. All other references are available in the registration dossier.

Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to skin sensitisation, exposure of workers, wide dispersive use, consumer use, high (aggregated) tonnage, mutagenicity and carcinogenicity, 2,3-epoxypropyl neodecanoate (CAS No 267621-45-5, EC No 247-979-2) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website 17 March 2015. The Competent Authority of Denmark (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA 17 March 2016.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation.

ECHA notified you of the draft decision and invited you to provide comments.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took into account your comments which were sent within the commenting period, and they are reflected in the Reasons (Appendix 1). By June 2016 you submitted update(s) of the registration dossier(s). The evaluating MSCA took the information in the updated registration dossier(s) into account, and it is reflected in the Reasons (Appendix 1).

Originally, the draft decision contained a request for a Carcinogenicity study (OECD 451). Following your comments, this request was removed from the current decision. It is, however, important to specify that the concern for carcinogenicity has not been clarified, but that the evaluating MSCA believes that clarification of this concern better can be done in the follow up phase once the information requested by this decision is available.

Proposals for amendment by other MSCAs and ECHA and referral to Member State Committee

The evaluating MSCA notified the draft decision to the Competent Authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposals for amendment to the draft decision, took them into account and modified the draft decision.
ECHA referred the draft decision, together with your comments, to the Member State Committee (MSC).

ECHA invited you to comment on the proposed amendment(s). You did not provide any comments on the proposed amendment(s).

MSC agreement seeking stage

Following the discussion in the Member State Committee a request on skin sensitisation was deleted. 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 Member State Committee reached a unanimous agreement on the draft decision during its 51st meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

   Further advice can be found at
http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.

Appendix 4: List of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

EC number: 247-979-2
CAS number: 26761-45-5
Public name: 2,3-epoxypropyl neodecanoate

This decision is addressed to the Registrant(s) of the above substance with active registrations according to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision in accordance with Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the registrant(s) that are addressees of the present decision is provided below: