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Helsinki, 11 September 2023

Addressee(s)
Registrant(s) of C12-14-alcohol MGE as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
17 May 2022

Registered substance subject to this decision ("the Substance")
Substance name: Oxirane, mono[(C12-14-alkyloxy)methyl] derivs.
EC/List number: 271-846-8

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***17 December 2024***.

Requested information must be generated using the Substance unless otherwise specified.

Information required from all the Registrants subject to Annex VII of REACH

1. Transgenic rodent somatic and germ cell gene mutation assays (triggered by Annex VII, Section 8.4., Column 2), as also requested below.

Information required from all the Registrants subject to Annex VIII of REACH

2. Transgenic rodent somatic and germ cell gene mutation assays (triggered by Annex VIII, Section 8.4., Column 2), as also requested below.

Information required from all the Registrants subject to Annex IX of REACH

3. Transgenic rodent somatic and germ cell gene mutation assays (Annex IX, Section 8.4.4.; test method: EU B.58./OECD TG 488) in transgenic rats, oral route, on the following tissue: stored duodenum samples from the OECD TG 488 study (2020) already performed with the registered substance.

The reasons for the request(s) are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressee(s) of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes or for different information requirements.

In the case of the same study requested under different Annexes, this is because some information requirements may be triggered at lower tonnage band(s). In such cases, only the reasons why the information requirement is triggered are provided for the lower

tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided.

In all cases, only one study is to be conducted; all registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements, you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also **update the chemical safety report, where** relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general requirements for testing and reporting new tests under REACH, see Appendix 4.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the request(s)
Appendix 2: Procedure
Appendix 3: Addressees of the decision and their individual information requirements
Appendix 4: Conducting and reporting new tests under REACH

[1] 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 for the request(s)

Contents

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Reasons related to the information under Annex VII of REACH

1. Transgenic rodent somatic and germ cell gene mutation assays

1 Under Annex VII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4, must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

1.1. Triggering of the information requirement

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 1997) with the Substance, which raise the concerns for gene mutations.

3 Therefore, the information requirement is triggered.

1.2. Information requirement not fulfilled

4 The information provided, its assessment and the specifications of the study design are addressed under request 3.

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Reasons related to the information under Annex VIII of REACH

2. Transgenic rodent somatic and germ cell gene mutation assays

5 Under Annex VIII, Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4, must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII or VIII, Section 8.4. The *in vivo* study must address the concerns raised by the *in vitro* study results, i.e., the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

2.1. Triggering of the information requirement

6 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 1997) with the Substance, which raise the concerns for gene mutations.

7 Therefore, the information requirement is triggered.

2.2. Information requirement not fulfilled

8 The information provided, its assessment and the specifications of the study design are addressed under request 3.

Reasons related to the information under Annex IX of REACH

3. Transgenic rodent somatic and germ cell gene mutation assays

9 An appropriate *in vivo* mammalian somatic cell genotoxicity study is an information requirement under Annex IX, Section 8.4.4., if there is a positive result in any of the *in vitro* studies referred to in Annex VII or VIII, Section 8.4.

3.1. Triggering of the information requirement

10 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 1997) with the Substance, which raise the concerns for gene mutations.

11 Therefore, the information requirement is triggered.

3.2. Information provided

12 You have provided:

(i) Three *in vivo* cytogenicity studies (key OECD TG 474, 2009; supporting non-guideline *in vivo* micronucleus study, 1977; supporting OECD TG 475, 1979) with the Substance that show negative results;
(ii) An *in vivo* gene mutation study (OECD TG 488, 2020) with the Substance that shows negative results in the liver and the glandular stomach.

3.3. Assessment of the information provided

3.3.1. Studies (i) not adequate for the information requirement

13 (Eco)toxicological studies must comply with a recognised test method (Article 13(3) of REACH). To address the specific concern raised by the *in vitro* positive results, an *in vivo* somatic cell genotoxicity study must be conducted according to the OECD TG 488 or 489, as indicated in the Guidance on IRs and CSA, Section R.7.7.6.3. Such study must cover the key parameters of the corresponding OECD test guideline (Article 13(3) of REACH).

14 Studies (i) are *in vivo* cytogenicity studies investigating chromosomal aberration. These studies are not *in vivo* somatic cell genotoxicity studies addressing concerns for gene mutations.

15 Therefore, the information provided (studies (i)) does not cover the specification(s) required by the OECD TG 488 or 489.

16 Based on the above, studies (i) are not adequate for the information requirement.

3.3.2. The provided study (ii) does not meet the specifications of the test guideline(s)

17 To fulfil the information requirement, a study must comply with the OECD TG 488 (Guidance on IRs and CSA, Section R.7.7.3.1, Table.7.7-3) (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) the highest dose studied is the maximum tolerated dose (MTD), i.e., the highest dose that is tolerated without evidence of toxicity (e.g., body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia);

b) the concurrent negative control data are within lower and upper bound limits of the distribution of the laboratory's historical negative control database;
c) in cases where the response is not clearly negative or positive, or in the case of a positive result at the only dose used in a limit test, further investigations of the existing experiments may be necessary in order to assist in establishing the biological relevance of a result (e.g. a weak or borderline increase).

18 In study (ii):

a) you did not demonstrate that the highest dose studied was the maximum tolerated dose. In the full study report for study (ii), you indicate that dose levels for the mutation assay part of the study were selected based on the 5-day range-finding study, which showed a lack of weight gain in the high dose group (750 mg/kg bw/d) even if this high dose was well tolerated. However, no body weight depression or other signs of study-limiting toxicity were observed in the actual mutation assay part of the study, and the mean body weight of the high dose group was only slightly lower than that of the vehicle control group (8.6% lower) at the end of the experiment (on day 31). In the comments to the draft decision, you further justify the dose selection based on site-of-contact effects observed in previously conducted repeated-dose studies in Han Wistar rats. In particular, you indicate that four out of ten animals treated at 750 mg/kg bw/day showed thickening of the non-glandular region of the stomach in a 14-day range-finding study (2018) provided with your comments, and that lesions were observed in the stomachs of males and females given 300 or 750 mg/kg bw/day in a 90-day repeated dose study (OECD TG 408, 2019). Although you acknowledge the differences in duration, substance concentration, administered volume and tested strains between the different studies, you argue that the high-dose level in the OECD TG 488 study (2020) was set at 750 mg/kg bw/day to minimize the anticipated site-of-contact effects in the stomach tissues. However, from the OECD TG 488 (2020) full study report, ECHA notes that no lesions in the glandular stomach were observed in any of the control or treated animals during the gross necropsy examination. This absence of site-of-contact effects contradicts your justification and does not support your dose selection.
b) the concurrent negative control data (mean mutant frequency = 17.6 x 10^{-6}) fall outside the lower and upper bound limits of the distribution of the laboratory's historical negative control database for the glandular stomach (95% Control limits are 24.6 x 10^{-6} and 43.8 x 10^{-6}). In the comments to the draft decision, you acknowledge this finding.
c) you conclude that "*Treatment with Oxirane at dose levels up to 750 mg/kg/day did not cause biologically significant increases in mutant frequency (MF) at the cII gene in the liver or glandular stomach of* [redacted] *male rats*". However, ECHA notes the following issues with your interpretation of the results for the glandular stomach:
   - There is a statistically significant increase in mutant frequency at the top dose (mean mutant frequency of 30.3 x 10^{-6} at 750 mg/kg bw/d, *vs.* 17.6 x 10^{-6} for the vehicle control);
   - a dose-related increase in mutant frequency was observed with mean values of 18.5, 23.4 and 30.3 x 10^{-6} at 100, 250 and 750 mg/kg bw/d, respectively.

   According to OECD TG 488, the above observations meet two out of the three evaluation criteria for a clearly positive result (the third criterion on a mutant frequency increase outside the upper bound limit of the historical negative control distribution is not met). Therefore, the glandular stomach results are not clearly negative or positive and further investigations of the existing experiment are necessary, like analysis of duodenum as another site-of-contact tissue.

19 Based on the above, the information provided does not cover the specification(s) required by the OECD TG 488.

20 Therefore, the information requirement is not fulfilled.

21 As the above acceptability criteria (a) and (b) of OECD TG 488 are not met, ECHA has some doubts on the validity of study (ii). In particular, signs of dose-limiting toxicity are not obvious from the OECD TG 488 study or related dose-range finding study provided, and higher doses than 750 mg/kg bw/d could have been tested according to OECD TG 488.

22 However, as a gene mutation response was still observed in the glandular stomach and the results in this tissue are inconclusive, ECHA considers that further investigations are required to ensure a sufficient evaluation of the gene mutation potential of the Substance at site of contact in the gastro-intestinal tract, like analysis of the duodenum.

23 In the comments to the draft decision, you indicate that the analysis of the stored duodenum samples is possible but highlight some technicalities for the design of this analysis, as further described below.

3.4. Test selection

24 According to the Guidance on IRs & CSA, Section R.7.7.6.3 the Transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) is suitable to follow up a positive *in vitro* result on gene mutation.

25 In your dossier, you provided an OECD TG 488 study (2020) with the Substance.

3.5. Specification of the study design

3.5.1. TGR assay

26 According to the test method OECD TG 488, the test must be performed in transgenic mice or rats. The provided OECD TG 488 study (2020) with the Substance was performed in transgenic rats.

27 Also, according to the test method OECD TG 488, the test substance is usually administered orally. In the provided OECD TG 488 study (2020), the Substance was administered by oral gavage.

28 Based on OECD TG 488 (30 June 2022), a 28+28d regimen must be followed, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals. Tissue collection three days after the final treatment (i.e., 28+3d regimen), as it was recommended in previous versions of the OEC TG 488, remains a valid sampling time when no germ cell data is needed. The provided OECD TG 488 study (2020) with the Substance was performed following the 28+3d regimen, which is considered suitable for the analysis of somatic tissues.

29 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver, as slowly proliferating tissue and primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. Furthermore, the duodenum must then be analysed, only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

30 Based on the information available in your dossier, the OECD TG 488 study (2020) you conducted with the Substance included collection of the duodenum samples and those were stored for future use. As the results from this study were negative in the liver and inconclusive in the glandular stomach, further studies at another site of contact are required to enable a conclusion on the gene mutation concern identified *in vitro*.

31 Therefore, you must use the stored duodenum samples to perform this additional analysis.

32 In the comments to the draft decision, you agree with the possibility to perform the requested analysis. However, you indicate that it will be done in a different laboratory, as the one that conducted the initial study (OECD TG 488, 2020) ceased its activity. Therefore, you intend to analyse the negative control data of both laboratories to compare their performance and use these data as reference for the requested study. In addition, you highlight that a different positive control from recently banked duodenum samples of ENU treated animals will be used, as the original positive control samples are not accessible anymore.

33 ECHA acknowledges your intention to make comparisons of the negative control data with the historical control data ranges of both laboratories to ensure that the data generated are consistent with existing data. Moreover, ECHA agrees that, according to OECD TG 488, tissues from previous positive control treated animals can be used instead of concurrent positive controls, provided that the testing laboratory has demonstrated proficiency in the conduct of the test and has established a historical control range for the tissue under investigation

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).
Guidance for monomers and polymers; ECHA (2012).
Guidance on intermediates; ECHA (2010).
All guidance documents are available online: https://echa.europa.eu/guidance-documents/guidance-on-reach

Read-across assessment framework (RAAF)

RAAF, 2017 Read-across assessment framework (RAAF); ECHA (2017).
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs; ECHA (2017).

The RAAF and related documents are available online: https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

***OECD Guidance documents* (OECD GDs)**

OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present. In particular, a subsequent germ cell genotoxicity study (TGR/OECD TG 488) may be required under Annex IX, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 24 May 2022.

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

ECHA took into account your comments and did not amend the request(s) or the deadline. The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended to 12 months to take into account currently longer lead times in contract research organisations.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Addressee(s) of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- the information specified in Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. Test methods, GLP requirements and reporting

(1) Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

(2) Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

(3) Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[2].

(4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(1) Selection of the Test material(s)
The Test Material used to generate the new data must be selected taking into account the following:

- the variation in compositions reported by all members of the joint submission,
- the boundary composition(s) of the Substance,
- the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

(2) Information on the Test Material needed in the updated dossier

- You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
- The reported composition must include the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note, Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also any constituents that have harmonised classification and

[2] https://echa.europa.eu/practical-guides

labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods,

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers (https://echa.europa.eu/manuals).