

Helsinki, 10 October 2023

**Addressee**

Registrant of EC\_244-435-6 as listed in Appendix 3 of this decision

**Date of submission of the dossier subject to this decision**

25/10/2017

**Registered substance subject to this decision ("the Substance")**

Substance name: Bis(2,3-epoxypropyl) cyclohex-4-ene-1,2-dicarboxylate

EC number/List number: 244-435-6

**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 **19 October 2026**.

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

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

1. *In vitro* micronucleus study also requested below (triggered by Annex VII, Section 8.4., Column 2)
2. *In vivo* genetic toxicity study also requested below (triggered by Annex VII, Section 8.4., column 2)
3. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202)
4. Growth inhibition study on aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3/OECD TG 201)

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

5. *In vitro* micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487)  
The aneugenic potential of the Substance must be assessed with an additional control group for aneugenicity on top of the control group for clastogenicity, if the Substance induces an increase in the frequency of micronuclei
6. *In vivo* genetic toxicity study (Annex VIII, Section 8.4., column 2) to be selected according to the following specifications:
  - a) If the results of the *in vitro* micronucleus study requested under 5. are **negative**:  
Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488) in transgenic mice or rats, oral route, on the following tissues: liver and glandular stomach; duodenum must be harvested and stored for up

to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

**OR**

*In vivo* mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

- b) If the results of the *in vitro* micronucleus study requested under 5. are **positive**:

*In vivo* mammalian alkaline comet assay (test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route. For the comet assay the following tissues must be analysed: liver, glandular stomach and duodenum. For the micronucleus test:

- the aneugenic potential of the Substance must be assessed by using a centromere staining technique if the substance induces an increase in the frequency of micronuclei in the OECD TG 474, unless the aneugenic potential has been conclusively investigated in the *in vitro* micronucleus study requested under Section 5.;
- target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

7. Short-term repeated dose toxicity (28 days) (Annex VIII, Section 8.6.1.) by oral route, in rats, to be combined with the screening for reproductive/developmental toxicity requested below

8. Screening study for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.64/OECD TG 422) by oral route, in rats

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 of the decision and its 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. 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. 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.

### **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<sup>1</sup> 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

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<sup>1</sup> 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)**

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## Reasons common to several requests

### 0.1. Read-across adaptation rejected

1 You have adapted the following standard information requirements by using grouping and read-across approach under Annex XI, Section 1.5.:

- In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- Short-term repeated dose toxicity (28 day), (Annex VIII, Section 8.6.1.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.)
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

2 ECHA has considered the scientific and regulatory validity of your read-across approach(es) in general before assessing the specific standard information requirements in the following sections.

3 Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

4 Additional information on what is necessary when justifying a read-across approach can be found in the Guidance on IRs and CSA, Chapter R.6. and related documents (RAAF, 2017; RAAF UVCB, 2017).

#### 0.1.1. Predictions for (eco)toxicological properties

5 You provide a read-across justification document in IUCLID Section 13.2.

6 You define the source substance in your read-across justification document as "a UVCB comprised of six main structures" which has "One main component typically represents [REDACTED]% of the composition, which has the constituent ([REDACTED]%) in the target substance bis(2,3-epoxypropyl) cyclohex-4-ene-1,2-dicarboxylate". You further claim that "The target substance contains one other constituent that can be present between [REDACTED]% which is also comparable to a constituent present in the source material at [REDACTED]%".

7 In the read-across justification document you name the source substance as bis(2,3-epoxypropyl) cyclohexane-1,2-dicarboxylate, which corresponds to the main constituent of the UVCB substance described above.

8 However, in the test material information under the relevant information requirements in IUCLID you identify the source substance as: [REDACTED], which is a UVCB substance. You also reported the major constituents, among which bis(2,3-epoxypropyl) cyclohexane-1,2-dicarboxylate. Therefore, ECHA considers that you intend to predict the properties of the Substance from information obtained from this test material as your "source substance".

9 You provide the following reasoning in your read-across justification document for the prediction of (eco)toxicological properties from the source substance: "*The read-across hypothesis is that the organism is not exposed to common compounds but rather, as a result of structural similarity, that different compounds have similar (eco)toxicological and fate properties. These compounds may be the source and target substances themselves or one or more of their (bio)transformation products. It is explained, on a mechanistic level,*

*why the similar properties are expected although the test organism is exposed to different compounds. This read-across hypothesis can also be used to predict the absence of effects".*

10 ECHA understands that your read-across hypothesis assumes that different compounds have the same type of effects. You predict the properties of your Substance to be quantitatively equal to those of the source.

11 ECHA has analysed the provided information and has identified the following issues:

*0.1.1.1. Missing supporting information to compare toxicological properties of the substance(s)*

12 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6., Section R.6.2.2.1.f.).

13 Supporting information must include, among others, bridging studies to compare properties of the substances.

14 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effects. In this context, relevant, reliable and adequate information allowing to compare the properties of the substances is necessary to confirm that the substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance.

15 In order to support your claim that the Substance and source substance have similar properties for the endpoints under consideration, you refer to studies relating to the acute toxicity, skin irritation, eye irritation, skin sensitisation, and in vitro gene mutation properties of the Substance and the source substance. While this information may provide support that the substances behave similarly for these toxicological properties, these studies do not inform on the cytogenicity, systemic and reproductive toxicological properties of the Substance and source substance. Therefore, this information is not considered as relevant to support your read-across hypothesis.

16 Based on above, the available data set does not provide adequate supporting information to support your claim of similarity in toxicological properties. Consequently, no reliable comparison of the properties of the Substance and the source substance can be made.

*0.1.1.2. Missing supporting information to compare ecotoxicological properties of the substance(s)*

17 Annex XI, Section 1.5. requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide supporting information to scientifically justify the read-across explanation for prediction of properties. The set of supporting information should strengthen the rationale for the read-across in allowing to verify the crucial aspects of the read-across hypothesis and establishing that the properties of the Substance can be predicted from the data on the source substance(s) (Guidance on IRs and CSA R.6., Section R.6.2.2.1.f.).

18 Supporting information must include bridging studies supporting information to compare properties of the substances.

19 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substance(s) cause the same type of effect(s). In this context, relevant,

reliable and adequate information allowing to compare the properties of the substance(s) is necessary to confirm that the substances cause the same type of effects. Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

20 In the read-across justification document, you claim that the Substance and the source substance have similar ecotoxicological properties.

21 In the dossier you have provided the following ecotoxicological studies:

- Short-term toxicity study on fish with the Substance;
- Short-term toxicity study on Daphnia with the source substance;
- Algae growth inhibition study with the source substance.
- In addition, you refer in the data matrix to the results of short-term toxicity study on fish with the source substance, however you have not provided the study in the registration dossier.

22 As the short-term toxicity study on fish with the source substance, included in the data matrix of the read-across justification document, is not provided in the dossier, ECHA cannot perform an independent assessment of its reliability. Therefore, the data set reported in the technical dossier does not include relevant, reliable and adequate information for the Substance and the source substance to support your read-across hypothesis.

23 In the absence of such information, you have not established that the Substance and the source substance(s) are likely to have similar properties. Therefore you have not provided sufficient supporting information to scientifically justify the read-across.

#### *0.1.2. Conclusion on the read-across approach*

24 Based on the above, you have not established that relevant properties of the Substance can be predicted from data on the source substance. Your read-across approach under Annex XI, Section 1.5. is rejected.

25 In the comments to the draft decision you reiterate your adaptation of the information requirement according to Annex XI, Section 1.5. You present a strategy relying on the generation of additional supporting information on the Substance and on the analogue substance.

26 You intend to perform the following '*additional bridging studies*':

- hydrolysis as function of pH (OECD TG 111) on the Substance and the analogue substance;
- short-term toxicity testing on aquatic invertebrates (OECD TG 202) on the Substance and the analogue substance;
- combined repeated dose toxicity study with the reproductive/developmental toxicity test (OECD TG 422) with the inclusion of additional Pig-a analysis (OECD TG 470) on the Substance.

27 You indicate your intention to provide a revised read-across justification including this information in a future update of your registration dossier.

28 ECHA acknowledges your intentions to improve the (eco)toxicological profile of the Substance and your plans to refine your read-across approach. As indicated in your comments, this strategy relies essentially on data which is yet to be generated, therefore no conclusion on the compliance can currently be made. You remain responsible for complying with this decision by the set deadline.

## Reasons related to the information under Annex VII of REACH

### 1. In vitro micronucleus study

29 Under Annex VII, Section 8.4., Column 2, an *in vitro* study referred to in Annex VIII, Section 8.4.2, must be performed if there is a positive result in the *in vitro* gene mutation study in bacteria.

#### 1.1. Triggering of the information requirement

30 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (2014).  
31 Therefore, the information requirement is triggered.

#### 1.2. Information requirement not fulfilled

32 The information provided in your dossier and in your comments on the draft decision, its assessment and the specifications of the study design are addressed under request 5.

### 2. In vivo mammalian genetic toxicity study

33 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.

#### 2.1. Triggering of the information requirement

34 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (2014) which raise the concern for gene mutation.  
35 Therefore, the information requirement is triggered.

#### 2.2. Information requirement not fulfilled

36 The information provided in your dossier and in your comments on the draft decision, its assessment and the specifications of the study design are addressed under request 6.

### 3. Short-term toxicity testing on aquatic invertebrates

37 Short-term toxicity testing on aquatic invertebrates is an information requirement under Annex VII to REACH (Section 9.1.1.).

#### 3.1. Information provided



38 You have adapted this information requirement by using Annex XI, Section 1.5. (grouping of substances and read-across approach) based on experimental data from the following substances:

- (i) a short-term toxicity study on *daphnia magna* (1992) with the source substance.

*3.2. Assessment of the information provided*

*3.2.1. Read-across adaptation rejected*

39 As explained in Section 0.1. Reasons common to several requests, your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected. In addition, ECHA identified endpoint specific issue(s) with the proposed prediction of (eco)toxicological properties, addressed below.

*3.2.1.1. Inadequate or unreliable study on the source substance(s)*

40 Under Annex XI, Section 1.5., the results to be read across must, among others, have an adequate and reliable coverage of the key parameters addressed in the test guideline for the corresponding study that shall normally be performed for a particular information requirement, in this case OECD TG 202, and meet the specifications of OECD GD 23 if the substance is difficult to test. Therefore, the following specifications must be met:

41 *Characterisation of exposure*

- a) analytical monitoring must be conducted. A reliable analytical method for the quantification of the test material in the test solutions with reported specificity, recovery efficiency, precision, limits of determination (i.e. detection and quantification) and working range must be available;
- b) the effect values can only be based on nominal or measured initial concentration if the concentration of the test material has been satisfactorily maintained within 20 % of the nominal or measured initial concentration throughout the test (see also Guidance on IRs and CSA, Section R.7.8.4.1.);

42 In study (i):

*Characterisation of exposure*

- c) no analytical monitoring of exposure was conducted
- d) the reported effect values are based on nominal concentrations.

43 Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results. More specifically, the test material is difficult to test due to its UVCB nature and you have not demonstrated that the substance is stable in test media during the test period.

44 Furthermore, there are indications in your dossier that the test item may not be stable in test media during the exposure period, as reported in IUCLID 6.1.5 (growth inhibition study on algae). Specifically, in the growth inhibition study on algae the measured concentrations of the test material decreased to ca. 70% of the nominal concentration.

45 Therefore, in the absence of analytical monitoring of the test concentrations (a) you have not demonstrated that the exposure concentrations were kept within 20% variation of the nominal concentration (b) hence, that you can report the effect values based on nominal concentration. Taken together, based on the above deficiencies, the hazard may be underestimated.

46 On this basis, the specifications of OECD TG 202 are not met.

47 Therefore, the study submitted in your adaptation, as currently reported in your dossier, does not provide an adequate and reliable coverage of the key parameter(s) of the corresponding OECD TG.

48 Therefore, the information requirement is not fulfilled.

### *3.3. Study design and test specifications*

49 The Substance is difficult to test due to its UVCB nature, and, as indicated by you in the dossier, due to the hindered solubility

50 Specifically, you indicate in IUCLID 6.1.1: Short-term toxicity to fish "Preliminary solubility work conducted indicated that the test item [the Substance] was practically insoluble in water using traditional methods of preparation e.g. ultrasonication.

51 Based on this information the test item was categorized as being a 'difficult substance' as defined by the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (OECD 2000)". This is supported by the robust study summary where you report that a 100% v/v saturated solution of the Substance, requiring 24h stirring and filtration steps, corresponded to 79 mg/L time-weighted mean measured concentration.

52 OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations.

53 Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results.

54 If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120% of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 202.

55 In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

56 In the comment to the draft decision, you agree to perform the requested study.

## **4. Growth inhibition study aquatic plants**

57 Growth inhibition study on aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

### *4.1. Information provided*

58 You have adapted this information requirement by using Annex XI, Section 1.5. (grouping of substances and read-across approach) based on experimental data from the following substances:

(i) inhibition study on aquatic plants/algae (2012) with the source substance.

### *4.2. Assessment of the information provided*

#### *4.2.1. Read-across adaptation rejected*

59 As explained in Section 0.1. Reasons common to several requests, your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected.

60 In the comments to the draft decision, you indicate your intention to improve your Annex XI, Section 1.5. (grouping of substances and read-across approach) adaptation. As explained in Section 0.1, as this strategy relies essentially on data which is yet to be generated, no conclusion on the compliance can currently be made.

61 Therefore, the information requirement is not fulfilled.

*4.3. Study design and test specifications*

62 The OECD TG 201 specifies that, for difficult to test substances, the OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in "Study design and test specifications" under request 3.

## Reasons related to the information under Annex VIII of REACH

### 5. In vitro micronucleus study

63 An in vitro mammalian chromosomal aberration study or an in vitro mammalian micronucleus study is an information requirement under Annex VIII, Section 8.4.2.

#### 5.1. Information provided

64 You have adapted this information requirement by using Annex VIII, Section 8.4., Column 2, in conjunction with Annex XI, section 1.5. (read-across).

65 To support the adaptation, you have provided the following information:

(i) *You state that "One in-vivo mutagenicity assays was performed: Micronucleus test in bone marrow cells of the mouse, the results of the study were negative and the test item (source) and therefore target is also concluded to have no significant mutagenic potential in-vivo."*

(ii) Micronucleus assay *in vivo* (2012) with the source substance

#### 5.2. Assessment of the information provided

66 We have assessed the provided information and identified the following issues:

5.2.1. *The provided adaptation does not meet the criteria of Annex VIII, Section 8.4., Column 2 as the read-across adaptation is rejected*

67 Under Annex VIII, Section 8.4., Column 2, the study referred to in Annex VIII, point 8.4.2, does not need to be conducted if adequate data from an in vivo micronucleus or in vivo chromosomal aberration study are available. The Guidance on IRs and CSA, Section R.7.7.6.3 and Table R.7.7-3 clarifies that such an in vivo somatic cell cytogenicity test must be performed according to the OECD TG 474 or 475.

68 The study (ii) provided, is described as in vivo mammalian erythrocyte micronucleus test (OECD TG 474), performed with the source substance.

69 As explained in Section 0.1. Reasons common to several requests, your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected.

70 Therefore, as there is no reliable in vivo micronucleus study, the Annex VIII, Section 8.4., column 2 criteria are not met and your adaptation is rejected.

71 In the comments to the draft decision, you indicate your intention to improve your Annex XI, Section 1.5. (grouping of substances and read-across approach) adaptation. As explained in Section 0.1, as this strategy relies essentially on data which is yet to be generated, no conclusion on the compliance can currently be made. On this basis, the information requirement is not fulfilled.

#### 5.3. Specification of the study design

72 According to the Guidance on IR & CSA, Section R.7.7.6.3., either the in vitro mammalian chromosomal aberration ("CA") test (test method OECD TG 473) or the in vitro mammalian cell micronucleus ("MN") test (test method OECD TG 487) can be used to investigate chromosomal aberrations in vitro.

- 73 However, while the MN test detects both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the CA test detects only clastogenicity, as OECD TG 473 is not designed to measure aneuploidy (see OECD TG 473, paragraph 2).
- 74 Therefore, you must perform the MN test (test method OECD TG 487), as it enables a more comprehensive investigation of the chromosome damaging potential *in vitro*.
- 75 Moreover, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen [1] (OECD TG 487, paragraphs 33 to 35).

#### 5.3.1. Assessment of aneugenicity potential

- 76 If the result of the MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance.
- 77 In line with the OECD TG 487 (paragraph 4), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).
- [1] According to the TG 487 (2016) 'At the present time, no aneugens are known that require metabolic activation for their genotoxic activity' (paragraph 34).

## 6. In vivo genetic toxicity study

- 78 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.

#### 6.1. Triggering of the information requirement

- 79 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (2014) which raise a concern for gene mutation.
- 80 Therefore, the information requirement is triggered.

##### 6.1.1. The provided studies do not address the concern raised by the positive *in vitro* result

- 81 The Guidance on IRs and CSA, Section R.7.7.6.3. clarifies that in order to justify that an *in vivo* somatic cell genotoxicity study does not need to be performed in accordance with Annex IX, Section 8.4., Column 2, the results of the available *in vivo* studies must address the specific concern raised by the *in vitro* positive result.
- 82 The study (ii) is described as micronucleus assay *in vivo*.
- 83 The provided *in vivo* study (ii) is not addressing the gene mutation concern raised by the positive *in vitro* gene mutation study.
- 84 Therefore, there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

- 85 In your comments on the draft decision you agree with ECHA that the positive results obtained in *in vitro* gene mutation studies warrant the conduct of an *in vivo* follow-up study.
- 86 You request the inclusion of the option to consider the mammalian erythrocyte Pig-a gene mutation assay (OECD TG 470), integrated into a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test as an alternative option to the Comet assay and the TGR assay.
- 87 You consider that the OECD TG 470 is a relevant test to assess *in vivo* mutagenicity and that it could be considered to follow-up on the positive *in vitro* results observed with the Substance. You highlight that "the Pig-a assay can be integrated into 28-day repeated dose toxicity studies" and that you point out that a 28-day repeated dose toxicity study is requested in this decision.
- 88 ECHA notes that as a result of the presence of epoxy functions, the Substance is very reactive and has the potential to cause site-of-contact toxicity. Therefore, the potential of the Substance to cause site-of-contact gene mutation *in vivo* needs to be investigated. This is reflected in the selection of organs/tissues specified for examination in the Comet assay or in the TGR assay with the inclusion of the glandular stomach and of the duodenum as site-of-contact tissues. While the mammalian erythrocyte Pig-a gene mutation assay is potentially suitable to follow-up on *in vitro* gene mutation positive results, this test cannot be used to measure mutations in site-of-contact tissues. Therefore, considering the reactivity of the Substance and the associated concern for gene mutation at the site-of-contact, the mammalian erythrocyte Pig-a gene mutation assay does not constitute an appropriate test method to follow-up on the positive *in vitro* results of the Substance.
- 89 Therefore, ECHA has not included the mammalian erythrocyte Pig-a gene mutation assay (OECD TG 470) as an alternative to the Comet assay or to the TGR assay in this decision.

## 6.2. Test selection

- 90 According to the Guidance on IRs & CSA, Section R.7.7.6.3 either the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) or the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) are suitable to follow up a positive *in vitro* result on gene mutation.
- 91 As explained above, under Request 4, in the dossier there is no adequate information from an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study, according to the requirements of Annex VIII, Section 8.4.2.
- 92 Therefore, by this decision, ECHA also requests an *in vitro* micronucleus study, which may raise a concern for chromosomal aberration in the case of positive results.
- 93 If there is also a concern for chromosomal aberration, the comet assay can be combined with an *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) in a single study (see OECD TG 489 para. 33; OECD TG 474 para. 37c; Guidance on IRs & CSA, Section R.7.7.6.3).
- 94 While the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations, the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy).
- 95 A combined study will therefore address both the identified concerns for chromosomal aberration as well as gene mutation.
- 96 The combined study, together with the results of the *in vitro* mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing *in vivo* mutagenicity and lack thereof.

97 Furthermore, the combined study can help limit the number of tests performed and the number of animals used while addressing the potential of the Substance to cause (structural and numerical) chromosomal aberrations as well as gene mutations.

98 Therefore, you must wait for the results of the *in vitro* test requested under request 5. and, depending on these results, to conduct either a) the TGR assay or Comet assay if the test results of request 4 are negative; or b) Comet assay combined with MN test if the test results of request 4 are positive. The deadline set in this decision allows for sequential testing.

### 6.3. Specification of the study design

a) Comet assay or TGR assay (if the test results of request under section 5. are negative)

#### 6.3.1. Comet assay

99 In case you decide to perform the comet assay, according to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified (OECD TG 489, paragraph 23).

100 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

101 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as sites of 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 genotoxicity at the site of contact in the gastro-intestinal tract.

or

#### 6.3.2. TGR assay

102 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

103 Also, according to the test method OECD TG 488, the test substance is usually administered orally.

104 Based on OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

105 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.

106 However, duodenum must be stored (at or below  $-70\text{ }^{\circ}\text{C}$ ) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed, only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

b) Comet assay combined with MN test (if the test results of request under section 5 are positive)

#### 6.3.3. Comet assay combined with MN test

107 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.

108 Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

109 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as sites of 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 genotoxicity at the site of contact in the gastro-intestinal tract.

110 The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen et al. 2011 [1]).

[1] Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Mutation Research* 722 7–19.

#### 6.3.3.1. Assessment of aneugenicity potential

111 If the result of the in vivo MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance unless the aneugenic potential has been conclusively investigated in the in vitro micronucleus study requested under Section 5. In line with the OECD TG 474 (paragraph 42), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

#### 6.3.3.2. Investigation of target tissue exposure

112 The applicable test method OECD TG 474 states that "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, a negative test result can be considered reliable only if "Bone marrow exposure to the test substance(s) occurred".

113 Therefore, to ensure that the data generated are adequate for hazard identification, you must take blood samples at appropriate times and measure plasma levels of the Substance and/or its metabolites (OECD TG 474, paragraph 40), unless exposure of the bone marrow



can be demonstrated through other means, e.g. by showing a depression of immature to mature erythrocyte ratio (OECD TG 474, paragraph 48).

- 114 If the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

#### 6.3.4. Germ cells

- 115 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483, depending on the concern raised by the substance) may still 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.

##### 6.3.4.1. Comet assay or Comet assay combined with MN test

- 116 In case you perform a comet assay, you may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

##### 6.3.4.2. TGR assay

- 117 In case you perform a TGR assay, you may collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below  $-70\text{ }^{\circ}\text{C}$ ). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

## 7. Short-term repeated dose toxicity (28 days)

- 118 A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII, Section 8.6.1.

### 7.1. Information provided

- 119 In your IUCLID dossier, sections 7.5.1. (Repeated dose toxicity: oral) you have provided the following information:

(i) Sub-chronic (90-days) repeated dose toxicity study (2017) with the source substance

- 120 While you have not provided a specific legal reference for an adaptation of this information requirement, ECHA understands that you use the provided study to adapt this information requirement according to Annex VIII, Section 8.6.1., Column 2 in conjunction with Annex XI, section 1.5. (read-across).

### 7.2. Assessment of the information provided

121 We have assessed the provided information and identified the following issues:

7.2.1. *The provided adaptation does not meet the criteria of Annex VIII, Section 8.7.1., Column 2 as read-across adaptation is rejected*

122 Under Annex VIII, Section 8.6.1., Column 2, Paragraph 1, Indent 1, the study may be omitted if a reliable sub-chronic (90 days) or chronic toxicity study is available or proposed by the registrant, provided that appropriate species, dosage, solvent and route of administration are used.

123 Study (i) is described as sub-chronic (90-day) repeated dose toxicity study, performed with the source substance.

124 As explained in Section 0.1. Reasons common to several requests, your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected.

125 Therefore, as there is no reliable sub-chronic toxicity study, your Annex VIII, Section 8.7.1., column 2 adaptation is rejected and the information requirement is not fulfilled.

### 7.3. Specification of the study design

126 When there is no information available neither for the 28-day repeated dose toxicity (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

127 The study design is addressed in request 8.

128 In your comments on the draft decision you agree to conduct the requested study. You request the possibility to integrate the mammalian erythrocyte Pig-a gene mutation assay (OECD TG 470) into the OECD TG 422 study. For the reasons presented under section 6 above, ECHA considers that the OECD TG 470 does not constitute an appropriate test method to follow-up on the positive *in vitro* results of the Substance and that it does not need to be added to the OECD TG 422 study.

## 8. Screening study for reproductive/developmental toxicity

129 A screening study for reproductive/developmental toxicity study (OECD 421 or OECD 422) is an information requirement under Annex VIII, Section 8.7.1., if there is no evidence from analogue substances, QSAR or *in vitro* methods that the substance may be a developmental toxicant.

### 8.1. Information provided

130 In your IUCLID dossier, sections 7.8.1. (reproductive toxicity) and 7.8.2. (developmental toxicity) you have provided the following information:

(i) Prenatal developmental toxicity study (2017) with the source substance

131 While you have not provided a specific legal reference for an adaptation of this information requirement, ECHA understands that you use it to adapt this information requirement

according to Annex VIII, Section 8.7.1., Column 2 in conjunction with Annex XI, section 1.5. (read-across).

*8.2. Assessment of the information provided*

132 We have assessed the provided information and identified the following issues:

*8.2.1. The provided adaptation does not meet the criteria of Annex VIII, Section 8.7., Column 2 as read-across adaptation is rejected*

133 Under Annex VIII, Section 8.7., Column 2, the study does not need to be conducted if a pre-natal developmental toxicity study (OECD TG 414) referred to in Annex IX, Section 8.7.2. is available.

134 The study (i) is a pre-natal developmental toxicity studies, performed with the source substance.

135 As explained in Section 0.1. Reasons common to several requests, your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5. is rejected.

136 Therefore, as there is no reliable pre-natal developmental toxicity study, your Annex VIII, Section 8.7.1., column 2 adaptation is rejected and the information requirement is not fulfilled.

*8.3. Specification of the study design*

137 When there is no information available neither for the 28-day repeated dose toxicity study (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

138 The information requirement for the 28-day repeated dose toxicity study is not fulfilled for the reasons explained under request 7.

139 Therefore, a study according to the test method EU B.64/OECD TG 422 must be performed in rats.

140 As the Substance is a liquid, the study must be conducted with oral administration of the Substance (Annex VIII, Section 8.7.1, Column 1).

141 Therefore, the study must be conducted in rats with oral administration of the Substance.

142 In your comments on the draft decision you agree to conduct the requested study. You request the possibility to integrate the mammalian erythrocyte Pig-a gene mutation assay (OECD TG 470) into the OECD TG 422 study. For the reasons presented under section 6 above, ECHA considers that the OECD TG 470 does not constitute an appropriate test method to follow-up on the positive *in vitro* results of the Substance and that it does not need to be added to the OECD TG 422 study.

## 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.

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

The compliance check was initiated on 01 February 2022.

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

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

ECHA took into account your comments and did not amend the requests.

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 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;

<b>Registrant Name</b>	<b>Registration number</b>	<b>Highest REACH Annex applicable to you</b>
[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<sup>2</sup>.
- (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**

- (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 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 all constituents of each Test Material and their concentration values.

With that detailed information, ECHA can confirm whether the Test Material is relevant for the Substance.

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>).

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<sup>2</sup> <https://echa.europa.eu/practical-guides>